THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

High fiber rye foods decrease body weight and body fat and affect metabolic risk markers

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Food and Nutrition Science

Department of Biology and Biological Engineering

CHALMERS UNIVERSITY OF TECHNOLOGY

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Cover: Whole grain rye products from the RyeWeight study (illustrated by Kia Nøhr Iversen)

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Abstract

Overweight and obesity are major risk factors for development of non-communicable diseases, such as type-2 diabetes and cardiovascular disease, and increase the risk of early mortality. Diet and food consumption are among the most important factors in preventing and reversing overweight, obesity and their comorbidities. Whole grain has been associated with decreased risk of overweight and obesity in observational studies, but the results from interventions are inconsistent. This may be because very few interventions have been adequately designed for evaluation of the effects of whole grain on body weight management and these effects may differ between different whole grain sources. Rye is the cereal with the highest fiber content and has been suggested to be superior to wheat in inducing beneficial physiological effects with health implications, but large randomized controlled trials with well-characterized intervention foods are lacking. This thesis aimed to investigate the effects of rye-based cereals, compared with refined wheat-based cereals, on body weight loss and metabolic risk factors. Furthermore, the potential influence of subjective appetite and gut microbiota were investigated. High fiber rye-based cereal products were shown to induce greater reduction in body weight and body fat than corresponding refined wheat products after 6 and 12 weeks of intervention among overweight and obese men and women. No consistent effect of rye products on appetite response was found and the changes in body weight and body fat could not be linked to differences in subjective appetite or food intake. However, this may be due to methodological issues and warrants further research. Compared with refined wheat products, high fiber rye-based cereal products were shown to lower C-reactive protein, a marker of inflammation and a risk factor for cardiovascular disease, in two different populations. This effect was associated with reduction in abundance of certain bacteria in the gut that have previously been associated with decreased gut barrier integrity, suggesting that the effect of rye consumption on inflammation may, at least partly, be mediated through changes in gut microbiota composition and decreased gut permeability. In conclusion, the work included in this thesis suggests that replacing wheat-based cereals with high fiber rye-based cereals can aid the reduction of body weight and body fat, and reduce low-grade inflammation. These results can support the development of dietary guidelines and promote the development of healthier food products.

Keywords: rye, wheat, whole grain, cereals, dietary fiber, weight loss, appetite, gut microbiota, metabolic risk factors, randomized controlled trial.

List of publications

This thesis is based on the work contained in the following papers, which are referred to in the text by Roman numerals.

I. **KN. Iversen**, F. Carlsson, A. Andersson, K. Michaëlsson, M. Langton, U. Risérus, PM. Hellström and R. Landberg: A hypocaloric diet rich in high fiber rye foods causes greater reduction in body weight and body fat than a diet rich in refined wheat: a parallel randomized controlled trial in adults with overweight and obesity (the RyeWeight study). *Clinical Nutrition ESPEN*, 2021. DOI: 10.1016/j.clnesp.2021.07.007

II. **KN. Iversen**, J. Dicksved, C. Zoki, R. Fristedt, M. Langton and R. Landberg: The effects of high fiber rye foods, compared to refined wheat foods, on gut microbiota composition, plasma short chain fatty acids and implications for weight loss and metabolic risk factors (the RyeWeight study). Manuscript.

III. K. Xue, Y. Liu, **KN. Iversen**, M. Mazidi, Z. Qu, C. Dong, T. Jin, G. Hallmans, P. Åman, A. Johansson, G. He and R. Landberg: Impact of a fermented high-fiber rye diet on *Helicobacter pylori* and cardio-metabolic risk factors: a randomized controlled trial among *Helicobacter pylori*-positive Chinese adults. *Frontiers in Nutrition,* 2021, vol 7. DOI: 10.3389/fnut.2020.608623.

IV. **KN. Iversen**, D. Johansson, C. Brunius, T. Andlid, R. Andersson, M. Langton and R. Landberg: Appetite and subsequent food intake were unaffected by the amount of sourdough and rye in soft bread – a randomized cross over breakfast study. *Nutrients,* 2018, vol 10. DOI:10.3390/nu10111594.

Published papers not included in the thesis

- Y. Liu, K. Xue, **KN. Iversen**, Z. Qu, C. Dong, G. Hallmans, P. Åman, A. Johansson, G. He and R. Landberg: The effects of fermented rye products on gut microbiota and its association with metabolic factors in Chinese adults – an explorative study. *Food and Function*, 2021. DOI: 10.1039/D1FO01423D

- **KN. Iversen** and R. Landberg: Whole grains, gut microbiota and health – time to get personal? *Journal of Nutrition*, 2021, vol 151, issue 3, pp 459–461. DOI: 10.1093/jn/nxaa412

- AK. Eriksen, C. Brunius, M. Mazidi, PM. Hellström, U. Risérus, **KN. Iversen**, R. Fristedt, L. Sun, Y. Huang, NP. Nørskov, KEB. Knudsen, C. Kyrø, A. Olsen, A. Tjønneland, J. Dicksved and R. Landberg: Effects of whole-grain wheat, rye, and lignan supplementation on cardiometabolic risk factors in men with metabolic syndrome: a randomized crossover trial. *American Journal of Clinical Nutrition*, 2020, vol 1, pp 846–876. DOI: 10.1093/ajcn/nqaa026

- J. Suhr, S. Vuholm, **KN. Iversen** and M. Kristensen: Wholegrain rye, but not wholegrain wheat, lowers body weight and fat mass compared to refined wheat: a 6-week randomized study. *European Journal of Clinical Nutrition*, 2017, vol 71, pp 959–967. DOI: 10.1038/ejcn.2017.12

- S. Vuholm, D. Nielsen, **KN. Iversen**, J. Suhr, P. Westermann, L. Krych, JR. Andersen and M. Kristensen: Wholegrain rye and wheat affect some markers of gut health without altering the fecal microbiota in healthy overweight adults: a 6-week randomized trial. *Journal of Nutrition*, 2017, vol 147, pp 2067–2075. DOI: 10.3945/jn.117.250647

Submitted manuscript not included in the thesis

- **KN. Iversen**, K. Jonsson and R. Landberg: The effect of rye-based foods on postprandial insulin concentration: the rye factor. *Critical Reviews in Food Science and Nutrition* (under review).

Book contribution not included in the thesis

- S. Ibrügger, **KN. Iversen**, M. Kristensen and R. Landberg: "Whole grain and appetite" in Landberg and Scheers (ed.) *Wholegrain and Health*, 2nd edition, June 2021, Wiley-Blackwell Publishing. ISBN: 978-1-118-93943-7.

Contribution report

Papers I and II: Kia Nøhr Iversen (KNI) participated in the designing and planning of the study and in writing the protocol and ethical application, conducted the clinical trial, coordinated the sample analysis, analyzed and interpreted the data, and was responsible for writing the manuscripts.

Paper III: KNI analyzed and interpreted the data and was responsible for writing the manuscript.

Paper IV: KNI participated in conducting the appetite tests, analyzed and interpreted the data, and was responsible for writing the manuscript.

Abbreviations

AR	Alkylresorcinols
AUC	Area under the curve
BMI	Body mass index
CCK	Cholecystokinin
CRP	C-reactive protein
DXA	Dual energy x-ray absorptiometry
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
HDL	High-density lipoprotein
HS/HR	High sourdough/high rye
HS/LR	High sourdough/low rye
LDL	Low density lipoprotein
LS/HR	Low sourdough/high rye
LS/LR	Low sourdough/low rye
MS/MR	Medium sourdough/medium rye
NCD	Non-communicable disease
PYY	Peptide YY
SCFAs	Short chain fatty acids
VAS	Visual analogue scale

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1 INTRODUCTION

Overweight and obesity are major risk factors for development of non-communicable diseases (NCDs), such as type-2 diabetes, cardiovascular diseases and certain cancers, and it has been estimated that overweight and obesity may account for as many as 4 million deaths annually [1, 2]. Overweight and obesity can have negative effects on quality of life, as both conditions increase the risk of living with disease and disability [1], in addition to having huge costs for society [3]. It is estimated that 2 billion people are overweight worldwide, of whom more than 600 million are obese [2, 4]. In Sweden, over 50% of the adult population aged 16–84 years is overweight or obese and the numbers have been increasing over the past decade [5].

Overweight is defined as body mass index (BMI) ≥ 25 kg/m^2 and obesity as BMI ≥ 30 kg/m^2, though it has been suggested that the cut-offs in certain Asian-Pacific populations should be 1–2 kg/m^2 lower [6, 7]. Fundamentally, overweight and obesity are caused by an imbalance between energy intake and energy expenditure leading to accumulation of energy in the form of excess body fat [8]. However, the underlying causes of this imbalance are more complex and influenced by many factors, such as environment, genetics and medical conditions. Recently, other factors such as gut microbiota have also been suggested to be of importance [9]. However, diet and food consumption remain among the most important aspects for preventing and reversing overweight and obesity and their comorbidities [10, 11].

Carbohydrate-rich foods, including cereals, fruits, legumes and starchy vegetables, constitute the main energy source for the vast majority of populations worldwide and most dietary guidelines promote consumption of such foods [12, 13]. However, over the past decades, a decrease in carbohydrate intake has been seen, which may in part be a result of a rise in fad diets focusing on reducing carbohydrate intake and in some cases giving carbohydrates a bad reputation [14–16]. Carbohydrates include a wide range of molecules, from easily absorbed simple sugars to complex fibers resistant to enzymatic digestion and absorption, which have very different physiological effects and health implications. In line with this, recent research has shown that carbohydrate quality may be more important than carbohydrate quantity in determining the effects of carbohydrate consumption on human health [17]. Furthermore, carbohydrate-rich foods, such as cereals and legumes, have a much lower environmental impact than animal-based products, which can be considered a reason to encourage continued and even increased consumption of such foods [18].

Whole grain cereals are rich in dietary fiber and a range of bioactive components, and have consistently been associated with decreased risk of overweight, obesity and NCDs, making whole grain cereals a good source of high-quality carbohydrates [19, 20].

While whole grain consumption has consistently been associated with reduced risk of overweight and obesity in observational studies, there is a lack of data from appropriately designed intervention studies. Such studies are needed to establish a causal link between whole grain consumption and body weight management and to increase the understanding of the underlying mechanisms. Furthermore, causality must be established before specific health effects of whole grains can be communicated to consumers, for instance through so-called health claims authorized by the European Commission [21].

Among cereals, rye has the highest content of dietary fiber and has been suggested to induce beneficial physiological effects with implications for human health, such as enhanced satiety, decreased insulin response and reductions in blood lipids [22]. Enhanced satiety could in theory lead to reduced body weight, and satiety-enhancing foods could be a valuable tool in preventing and reversing overweight and obesity [23]. However, there is a need for studies testing the causal link between consumption of satiety-enhancing foods and long-term weight management, to understand how satiety-enhancing products may be used to prevent and reverse overweight and obesity. Recently, gut microbiota has emerged as a potential mediator of the health effects proposed to be induced by whole grain, and gut microbiota composition has in some cases been shown to determine the magnitude of weight loss induced by cereal fiber consumption [24, 25].

In summary, there is a need for well-designed interventions that can provide causal evidence for the effect of whole grain on body weight regulation, and prove a link between consumption of satiety-enhancing foods and body weight loss. Closing these gaps is important for the development of more effective dietary guidelines and for development and marketing of healthier food products, which can together improve the health of the population.

2 OBJECTIVES

The overall aim of the thesis was to evaluate the effects of high fiber rye-based foods compared with commonly consumed refined wheat-based foods on weight loss and metabolic risk factors, to aid reduction of the prevalence of overweight and obesity and their comorbidities.

The specific objectives were:

A. To investigate the effects of a high fiber whole grain rye-based diet, compared with a refined wheat diet, on weight loss (Paper I).
B. To explore the potential mechanistic effect of appetite on weight loss and investigate factors that may influence appetite response to typical rye-based cereal products (Papers I and IV).
C. To explore the potential influence of gut microbiota on weight loss in response to a whole grain rye-based diet (Paper II).
D. To investigate the effects on metabolic risk markers of a diet rich in high fiber rye-based foods (Papers I and III).

3 BACKGROUND

3.1 Cereals and human health

Cereals are major part of the diet in many areas of the world, but the consumption of cereals varies between different countries as regards both grain species and amounts [26]. Many countries in northern Europe, especially in Scandinavia, have a relatively high intake of rye-based cereal products [27, 28], whereas the cereal intake in southern Europe is dominated by wheat [29]. Oats and barley are consumed in several European countries, but to a lesser extent than wheat and rye. A large proportion of the rye consumed in northern Europe is in the form of whole grain, while many wheat products are typically based on refined wheat. Whole grain food intake is recommended by governmental authorities as part of the official dietary guidelines in many countries, but the exact recommendations differ somewhat between countries, ranging from encouragement to "choose whole grains whenever consuming cereals" to listing specific amounts of whole grain to be consumed on a daily basis [30].

There is currently no globally accepted definition of whole grains or whole grain products [31]. Attempts have been made to unify the definitions, and the Health Grain Forum, which is a European association of academic and industrial parties with an interest in cereals, proposed the following definition of whole grains: "*Whole grains shall consist of the intact, ground, cracked or flaked kernel after the removal of inedible parts such as the hull and husk. The principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact kernel*" [32]. Similar definitions have been adopted by other major regulatory bodies within the food sector [33, 34]. Though an official global definition regarding whole grain is lacking, there is a relatively strong consensus on what constitutes whole grain; the definition of a whole grain product varies more, though some suggestions for guidelines has been made [35]. Many products consist of a mixture of whole grain and refined grain and the actual whole grain content in products marked whole grain may vary substantially [35]. In some countries, attempts have been made to standardize the definition of a whole grain product, for instance through the use of a whole grain logotype by a public-private partnership in Denmark [36] and the Nordic "keyhole," which was originally developed by the Swedish National Food Agency in the 1980s and later adapted by the Nordic Council and used in several Nordic countries [37, 38].

Consumption of whole grains has consistently been associated with better health status in observational studies, whereas refined grains have in some studies been associated with increased disease risk and have in other studies not been associated with either increased or decreased risk of disease [39–41]. Whole grain consumption has for example been associated with decreased risk of developing type-2 diabetes, cardiovascular disease and certain cancers [39, 42–45]. Furthermore, whole grain consumption has been inversely associated with BMI and body weight, and seems to decrease the risk of overweight and obesity [46–49]. These

potential health effects have been attributed to many different components of whole grain cereals, but cereal fiber has been suggested to be one of the main contributors, although it has been shown to be difficult to separate the independent effects of bioactive compounds and those of dietary fiber *per se* [19, 50]. Dietary fiber refers to carbohydrate polymers from plants which are resistant to enzymatic digestion and absorption in the upper gastrointestinal (GI) tract and therefore pass undigested into the colon, where they to varying degree undergo digestion by the gut microbiota [51]. Dietary fiber is well established as an important part of a healthy diet, but the term covers a wide range of different fiber components from different sources. Their functionalities vary and thus it is likely that the specific health effects vary depending on the type and source of fiber [52–54]. The physiological effects of dietary fiber also range widely, from slowed gastric emptying and digestion of the food matrix in the upper GI tract and binding of various components hindering absorption, to acting as a substrate for the gut microbiota in the colon [52–55]. The gut microbiota has been suggested to be involved in the potential health effects of whole grain consumption [56]. However, whole grain-based interventions have shown a limited ability to alter the gut microbiota composition. This may be due to the fact that many studies have relatively low power and that changing only part of the diet (e.g., replacing habitual cereals with intervention cereals) may not be sufficient to induce major changes in the gut microbiota composition, especially in populations with high habitual intake of fiber-rich cereals [57–59].

Whole grains have a higher content of micronutrients and bioactive components than their refined counterparts. This is due to the fact that many such components in cereals are located in the outer bran and germ layers, which are often removed when grain is processed into refined cereal products [50]. There are also differences between different cereal sources; for instance, rye has an especially high content of lignans and benzoxazinoids and wheat has a high content of selenium [55, 60, 61].

3.2 Cereals and body weight management

Body weight management refers to interventions and strategies aiming at reaching and maintaining optimal body weight. The term optimal body weight often refers to the body weight and body composition that is optimal for promotion of health and prevention of disease and disability [62]. Body weight management can involve both weight gain and weight loss, as well as maintenance of a stable body weight [63], but in this thesis the main focus is on aspects related to weight loss.

A high intake of whole grain or cereal fiber has consistently been shown to be associated with lower BMI and lower risk of developing overweight and obesity in observational studies. A recent meta-analysis of cross-sectional cohort studies found an inverse association between whole grain consumption and BMI, i.e., study participants who reported a higher intake of whole grains had a lower BMI [64]. The same meta-analysis found an overall inverse association between whole grain intake and weight gain in prospective cohorts with a follow-

up time of 5–20 years [64]. Other meta-analyses have confirmed the inverse association between whole grain or cereal fiber consumption and obesity measures in prospective cohorts [65]. While observational studies enable studies of dietary patterns and their associations with health outcomes in large populations over long periods of time, they also suffer from measurement errors and potential confounding. Consumption of healthy foods, such as whole grains and cereal fiber, is often associated with a generally healthy diet and other healthy lifestyle patterns such as increased physical activity [28, 66, 67]. This means that it may be difficult to completely disentangle the potential health effects of each individual component of the lifestyle. A way to overcome this challenge and reduce the risk of confounding is to use randomized controlled intervention studies where participants are randomly assigned to specific treatment groups, such as one group eating whole grain products and another eating refined grain products [68].

3.2.1 Evidence from randomized intervention studies

While the results from observational studies show a clear link between whole grain intake or high cereal fiber intake and lower risk of overweight and obesity, the results from interventions are inconsistent [69, 70]. A meta-analysis from 2013 concluded that there was no beneficial effect on body weight on whole grain consumption, but indications of a small effect on body fat, and an updated meta-analysis from 2020 confirmed the finding of no effect of whole grains on obesity measures [69, 70]. The reason for the differential results when comparing observational studies and intervention studies may be related to the fact that very few of the interventions included in the meta-analyses were designed to investigate the effects of cereals on body weight. In fact, many were designed for investigation of outcomes sensitive to changes in weight and measures to prevent weight change were implemented in some studies. Table 3.1 summarizes randomized controlled cereal interventions, the majority of which were included in the aforementioned meta-analyses [69, 70]. Out of the 38 studies included in the table, only five were designed to investigate effects on obesity measures (body weight, body composition and abdominal fat mass) [71–75]. All the other studies were designed for other purposes and some even implemented measures to maintain stable weight throughout, ranging from simply instructing participants to maintain a stable weight [76–78] to regularly checking participants' weight and intervening if it changed [79, 80]. These are not optimal conditions for investigating the effects on body weight and might explain why most of the studies showed no effect of the intervention on body weight. Some studies that were not designed to investigate body weight did ask participants to replace their habitual cereals with intervention products on an *ad libitum* basis without further dietary restriction. These studies might add to the understanding of the effect of cereals on body weight regulation, which could be mediated through increased satiation or satiety and lower food intake following consumption of certain cereals. Nonetheless, it is still questionable to what extent most of the studies can be used to accurately address the question of cereal

consumption and weight management. It is unfortunate that this has not been highlighted to a greater extent in scientific reviews and meta-analyses.

Table 3.1 reveals a strikingly large variation in types and amounts of intervention foods used across studies. Many studies investigated mixtures of multiple cereal sources, but there were also many specifically focusing on wheat or oats. Different cereals vary greatly in nutritional composition. For instance, the fiber content varies from 6% in whole grain rice up to 20% in whole grain rye [22]. Furthermore, the fiber composition also varies greatly, with oats and barley having a high amount of soluble, viscous beta-glucans, whereas rye and wheat are dominated by a mixture of soluble fiber and primarily insoluble arabinoxylans [81]. These differences in composition could likely influence the physiological effects in the human body after consumption and there may be a need for greater distinction between different cereals to get a better understanding of the health effects of cereals. Moreover, processing affects the physiochemical properties of dietary fiber, which could also influence the physiological effects [82–84].

Rye has the highest fiber content of all cereals and has been suggested to be superior to other cereal types as regards health effects [22]. Rye has been suggested to exert unique effects on postprandial insulin secretion, a phenomenon that has been named the “rye factor,” which will be discussed in detail later. Furthermore, rye has consistently been shown to increase subjective satiety and decrease hunger and desire to eat in the postprandial phase, which could be hypothesized to lead to weight loss and improved weight maintenance in a longer-term perspective [23].

Table 3.1: *Overview of cereal-based randomized controlled studies reporting effects on body weight measures.*

	Design and duration	Subjects (% females)	Primary outcome	Cereal source	Intervention products	Background diet*	Results on body weight**
Van Horn 1988 [77]	P 2 arms 8 weeks	N: 236 (36%) A: 30–65	Blood lipids	WG: oat C: -	WG: Oatmeal (56 g/d, dry weight) C: no control product	Phase II AHA diet (weight stability)	WG: ↔ C: ↔ Group diff: NS
Van Horn 1991 [76]	P 2 arms 8 weeks	N: 80 (50%) A: 25–76 BMI: 26.2±3.4	Blood lipids	WG: Oat C: -	WG: Instant oats (56.7 g/d) C: no control product	Weight stability	WG: ↓ C: ↑ Group diff: NS
Johnston 1998 [85]	P 2 arms 6 weeks	N: 135 (40%) A: 40–70	Blood lipids	WG: oat RG: corn	WG: Cheerios (90 g/d) RG: Cornflakes (90 g/d)	NCEP Step One diet	WG: ↔ RG: ↔ Group diff: NS
Leinonen 2000 [78]	CO 2 arms 4 weeks	N: 40 (55%) A: 43±2.0 BMI: 20–32	Blood lipids	WG: rye RG: wheat	WG: Rye bread (min 20% of EI) RG: Wheat bread (min 20% of EI)	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Reynolds 2000 [86]	P 2 arms 4 weeks	N: 43 (51%) Y: 27–68	Blood lipids	WG: oat RG: corn	WG: Cheerios (43 g/d) RG: Corn-based puffed cereal (43 g/d)	AHA step-1 diet	WG: ↔ RG: ↔ Group diff: NS
Saltzman 2001 [87]	P 2 arms 8 weeks	N: 43 (53%) A: 18–30, 60–75 BMI: 20–35	Blood lipids and blood pressure	WG: oat C: -	WG: Meals incorporating oats (45 g/d) C: Meals not containing oats	Hypocaloric diet (all meals provided)	WG: ↓ C: ↓ Group diff: NS
Pereira 2002 [79]	CO 2 arms 6 weeks	N: 11 (55%) A: 21–65 BMI: 26–36	Insulin sensitivity	WG: mix RG: mix	WG: Meals containing WG products RG: Meals containing RG products	Weight stability (all meals provided)	WG: - RG: - Group diff: NS
Li 2003 [88]	CO 2 arms 4 weeks	N: 10 (100%) A: 20.4±1.3 BMI: 19.2±2.0	Blood lipids, blood glucose, fecal excretion	WG: barley RG: rice	WG: Meals with 30% rice replaced with barley RG: Meals with rice as main cereal source	Weight stability (all meals provided)	WG: - RG: - Group diff: NS
McIntosh 2003 [89]	CO 3 arms 4 weeks	N: 28 (0%) A: 40–65	Bowel function	WG1: rye WG2: wheat RG: wheat, rice	40 g crisp bread, 140 g soft bread and 50 g BC based on: WG1: WG rye WG2:WG wheat RG: RG wheat and rice	Low to moderate fiber intake from background diet	WG1: - WG2: - RG: - Group diff: NS
Karmally 2005 [90]	P 2 arms 11 weeks	N: 152 (67%) A: 30–70 BMI: < 38	Blood lipids	WG: oat RG: corn	WG: Cheerios (90 g/d) RG: Corn-based BC without soluble fiber (90 g/d)	Weight stability	WG: ↔ RG: ↔ Group diff: NS

Andersson 2007 [91]	CO 2 arms 6 weeks	N: 30 (73%) A: 35–70 BMI: 26–35	Insulin sensitivity, inflammation	WG: mix RG: mix	WG: Mixed WG cereal products (3,180 kJ/d) RG: Mixed RG cereal products (3,340 kJ/d)	Habitual diet	WG: ↑(BMI) RG: ↔ (BMI) Group diff: WG > RG
Bird 2008 [80]	CO 3 arms 4 weeks	N: 24 (54%) A: 31–66 BMI: 21–38	Bowel health	WG1: barley WG2: wheat RG: wheat, rice	WG1 and WG2: Mixed WG cereal products (240–255 g/d) RG: Mixed RG cereal products (240–255 g/d)	Low fiber background diet	WG1: ↔ WG2: ↔ RG: ↔ Group diff: NS
Katcher 2008 [71]	P 2 arms 12 weeks	N: 50 (50%) A: 24–63 BMI: ≥ 30	Body weight	WG: mix C: -	WG: 4–7 servings of WG/day (depending on energy needs) C: Instructed to avoid WG	Hypocaloric diet	WG: ↓ C: ↓ Group diff: NS
Brownlee 2010 [92]	P 3 arms 16 weeks	N: 316 (50%) A: 18–65 BMI: ≥ 25	Blood lipids	WG1: mix WG2: mix C: -	WG1: Mixed WG products equivalent of 60 g WG/d WG2: Wk 0–8: WG products equivalent of 60 g WG/d, wk 9–16: 120 g/d. C: no control product	Habitual diet	WG1: ↔ WG2: ↔ C: ↔ Group diff: NS
Giacco 2010 [93]	CO 2 arms 3 weeks	N: 15 (20%) A: 54.5±7.6 BMI: 27.4±3.0	Blood lipids	WG: wheat RG: wheat	WG: WG wheat bread, pasta, rusks, crackers RG: RG wheat bread, pasta, rusks, crackers	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Maki 2010 [94]	P 2 arms 12 weeks	N: 144 (78%) A: 20–65 BMI: 25–45	Blood lipids	WG: oat C: -	WG: Cheerios (80 g/d) C: no control product	Hypocaloric diet. Instructed to avoid foods high in viscous fiber.	WG: ↓ C: ↓ Group diff: NS
Tucker 2010 [95]	CO 2 arms 6 weeks	N: 28 (40%) A: 43–70 BMI: 19–25 and ≥ 30	Blood lipids	WG: wheat RG: wheat	WG: Sourdough WG wheat bread (contained smaller amounts of rye and oat in addition to wheat) RG: RG wheat bread	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Bodinham 2011 [96]	CO 2 arms 3 weeks	N: 14 (64%) A: 26 (mean) BMI: 22(mean)	Appetite	WG: wheat RG: wheat	WG: WG wheat bread rolls (2,328 kJ/d) RG: RG wheat bread rolls (2,337 kJ/d)	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
De Mello 2011 [97]	P 2 arms 12 weeks (fish/berry arm excluded)	N: 68 (49%) A: 40–70 BMI: 26–39	Inflammation, endothelial function	WG: wheat, rye C:-	WG: Bread (20–25% EI) and pasta (min 35 g/wk, dry weight) from WG wheat and rye. C: instructed to avoid WG	Habitual diet	WG: ↔ C: ↔ Group diff: NS

Ross 2011 [98]	CO 2 arms 2 weeks (WG/RG comp. only)	N: 17 (65%) A: 20–50 BMI: 19–28	Cardiovascular health	WG: wheat, oats, rice RG: wheat, rice, maize	WG: Full diet provided, containing mixed WG products RG: Full diet provided, containing mixed RG products	Weight stability (all meals provided)	WG: ↔ RG: ↔ Group diff: NS
Zhang 2011 [99]	P 2 arms 16 weeks	N: 202 (47%) A: 50 (mean) BMI: 26±3 (mean)	Blood glucose	WG: rice RG: rice	WG: Brown rice (*ad libitum*) RG: White rice (*ad libitum*)	Habitual diet	WG: ↓ RG: ↔ Group diff: NS
Kristensen 2012 [72]	P 2 arms 12 weeks	N: 79 (100%) A: 45–70 BMI: 27–37	Body weight	WG: wheat RG: wheat	WG: 62 g soft bread, 60 g pasta and 28 g biscuits per day (all WG) RG: 62 g soft bread, 60 g pasta and 28 g biscuits per day (all RG)	Hypocaloric diet	WG: ↓ RG: ↓ Group diff: NS
Giacco 2013 [100]	P 2 arms 12 weeks	N: 123 (53%) A: 40–65 BMI: 25–35	Glucose and insulin metabolism	WG: mix RG: mix	WG: Mixed WG products RG: Mixed RG products	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Wang 2013 [101]	P 2 arms 12 weeks	N: 57 (67%) A: 18+	Insulin sensitivity	WG: rice RG: rice	WG: Brown rice RG: White rice	Habitual diet	WG: ↓ RG: ↔ Group diff: WG < RG
Giacco 2014 [102]	P 2 arms 12 weeks	N: 54 (57%) A: 40–65 BMI: 25–35	Postprandial insulin	WG: mix RG: mix	WG: Mixture of WG products (60–80% of CHO intake) RG: Mixture of RG products (60–80% of CHO intake)	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Jackson 2014 [73]	P 2 arms 12 weeks	N: 50 (50%) A: 35–55 BMI: 25–42	Abdominal fat	WG: mix RG: mix	WG: 5.8–11.5 servings WG/day (depending on EI) RG: 5.8–11.5 servings RG/day (depending on EI)	6 wk weight stability + 6 wk hypocaloric (all meals provided)	WG: ↓ RG: ↓ Group diff: NS
Lappi 2014 [103]	CO 2 arms 4 weeks	N: 21 (-) A: 38–65 BMI: 19–30	Glucose metabolism	WG: rye C: wheat/rye	WG: WG rye bread (6–10 slices/day) C: RG wheat bread with added rye bran (6–10 slices/day)	Habitual diet	WG: ↔ C: ↔ Group diff: NS
Vitaglione 2014 [104]	P 2 arms 8 weeks	N: 68 (66%) A: 18+ BMI: 25–35	Ferulic acid bioavailability	WG: wheat RG: wheat	WG: Biscuits with shredded WG wheat (70 g/d) RG: Refined wheat crackers (33 g/d) and toast (27 g/d)	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Ampatzoglou 2015 [105]	CO 2 arms 6 weeks	N: 33 (64%) A: 40–65 BMI: 20–35	Metabolic risk markers	WG: mix RG: mix	WG: Mixed WG products as substitution of habitual cereals (> 80 g WG/d) RG: Mixed RG products as substation of habitual cereals (< 16 g WG/d)	Habitual diet	WG: ↔ RG: ↔ Group diff: NS

Kirwan 2016 [74]	CO 2 arms 8 weeks	N: 33 (82%) A: < 50 BMI: 28–40	Body composition	WG: mix RG: mix	WG: Mixed WG products incorporated into the diet (50 g/1,000 kcal) RG: Mixed RG products incorporated into the diet (50 g/1,000 kcal)	Weight stability (all meals provided)	WG: ↓ RG: ↓ Group diff: NS
Connolly 2016 [106]	CO 2 arms 6 weeks	N: 30 (63%) A: 23–64 BMI: 18–30	Gut microbiota composition	WG: oat RG: corn	WG: WG oat granola BC (45 g/d) RG: Flaked corn BC (45 g/d)	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Karl 2017 [107]	P 2 arms 6 weeks	N: 81 (40%) A: 40–65 BMI: 20–35	Immune function	WG: mix RG: mix	WG: WG products incorporated into meals (207±39 g WG/d) RG: RG products incorporated into meals (0 g WG/d)	Weight stability (all meals provided)	WG: ↔ RG: ↔ Group diff: NS
Kristensen 2017 [75]	P 2 arms 12 weeks	N: 178 (100%) A: 20–50 BMI: 27–34	Abdominal fat	WG: mix RG: mix	WG: Mixed WG products *ad libitum* RG: Mixed RG products *ad libitum*	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Suhr 2017 [108]	P 3 arms 6 weeks	N: 75 (56%) A: 30–65 BMI: 25–32	Gastrointestinal symptoms	WG1: rye WG2: wheat RG: wheat	WG1: WG rye products *ad libitum* WG2: WG wheat products *ad libitum* RG: RG wheat products *ad libitum*	Habitual diet	WG1: ↓ WG2: ↔ RG: ↔ Group diff: WG1 < RG
Schutte 2018 [109]	P 2 arms 12 weeks	N: 50 (38%) A: 45–70 BMI: 25–35	Cardiometabolic risk factors	WG: wheat RG: wheat	WG: 100 g WG bread and 33 g WG BC from WG wheat RG: 100 g RG bread and 33 g RG BC from refined wheat	Habitual diet	WG: ↔ RG: ↔ Group diff: NS
Roager 2019 [57]	CO 2 arms 8 weeks	N: 50 (64%) A: 20–65 BMI: 25–35	Insulin sensitivity and gut microbiota	WG: mix RG: mix	WG: Mixed WG products (*ad libitum*) RG: Mixed RG products (*ad libitum*)	Habitual diet	WG: ↔ RG: ↑ Group diff: WG < RG
Eriksen 2020 [59]	CO 2 arms 8 weeks	N: 40 (0%) A: 49–74 BMI: 30.5 (mean)	Glucose tolerance	WG1: rye WG2: wheat	WG crisp bread and BC based on WG rye or WG wheat (30% of EI)	Habitual diet	WG1: ↔ WG2: ↔ Group diff: NS
Åberg 2020 [110]	CO 2 arms 2 weeks	N: 31 (34%) A: 63±13 BMI: 33±7	Postprandial glucose	WG1: Mix WG2: Mix	WG1: WG products with finely milled WG WG2: WG products with coarser WG	Habitual diet	WG1: ↑ WG2: ↓ Group diff: WG2<WG1

Abbreviations: A: age in years; AHA, American heart association; BC, breakfast cereal; BMI, body mass index in kg/m^2; C, control; CHO, carbohydrate; CO, cross-over; d, days; diff, difference; EI, energy intake; g, grams; kcal, kilocalories; NCEP, National cholesterol education program; NS, no significant difference between groups; P, parallel; RG, refined grain; WG, whole grain; y, years. *Dietary instruction given to participants, besides consumption of intervention products. **If body weight is not reported, BMI is used instead (↔, no change in weight; ↑, increase in weight; ↓, decrease in weight; -, weight change within group not reported).

3.2.2 Effect of rye consumption on body weight

Due to its high fiber content and proposed satiety-enhancing effects, rye has been suggested to be superior to other cereals in generating weight loss [22]. One of the aforementioned meta-analyses included sub-group analyses to estimate the effects of individual cereals sources on body weight reduction [69]. No effect on body weight was found for rye, but at that time there were only two published studies reporting the effects of rye consumption on body weight [78, 89]. Both were cross-over studies with rather short intervention periods, which may not be optimal for evaluating changes in body weight. Since then, three additional studies have been published (Table 3.1) [59, 103, 108]. Two were similar to the previous studies, in the sense that they also followed a cross-over design, although one was of 8 weeks duration [59], where the others were 4 weeks [78, 89, 103]. Only one study used a parallel design [108]. This study included both a whole grain rye arm and a whole grain wheat arm, in addition to a refined wheat control arm, and could thus estimate the effects of whole grain *per se* and the effects of different sources of whole grain. This study was not designed to investigate weight loss. The participants were instructed to replace their habitual cereal products with intervention products in an *ad libitum* manner, rather than fixed amounts, and did not receive any other dietary restrictions. Their weight was not controlled during the study. Thus, even though the study was not designed for weight loss, it allowed for fluctuations in weight that might be induced by increased satiety or satiation due to the intervention products. Despite the relatively short duration of the study (6 weeks), the participants in the whole grain rye group lost 1.1 kg on average, a finding which differed significantly from what was seen in the participants in the refined wheat group, who gained 0.2 kg. The participants in the whole grain wheat group lost some weight (0.6 kg), but not as much as the participants in the whole grain rye group and the change in body weight did not differ significantly from that of the refined wheat group. This study suggested some degree of superiority of whole grain rye in inducing weight loss that was not solely explained by the fact that participants ate whole grain. These results should be interpreted with caution since the study was not designed to analyze weight loss and was of a relatively short duration and small size. However, this study did support the hypothesis that rye may have some unique properties that are beneficial for body weight management.

An effect of rye on body weight could, as mentioned above, be mediated through increased satiety, decreased hunger and lower subsequent energy intake, which will be discussed in detail later. It could also be influenced by other factors, such as fecal energy excretion or increased energy expenditure [107, 111]. Dietary fiber from cereals has been shown to bind nutrients in the GI tract and prevent absorption, which means that some of the energy consumed is excreted in the feces [107]. This could contribute to a negative energy balance, which would lead to body weight reduction.

In recent years, the gut microbiota has emerged as a potential mediator of weight loss induced by fiber-rich diets and it has been suggested that some of the inter-individual variations in weight loss in response to a fiber-rich diet may be explained by differences in the

gut microbiota composition [24, 112–114]. A *post hoc* analysis of the previously mentioned study that found reduction in body weight in response to a whole grain rye diet showed that the weight loss in response to the whole grain-based diets correlated with abundance of *Prevotella* and that participants with high abundance of *Prevotella* lost more weight than participants with low abundance [24]. However, the whole grain rye and the whole grain wheat groups were pooled for the *post hoc* analysis, so it is unknown if these correlations differed between the cereal sources. Obesity has been associated with lower abundance of *Bifidobacteria* and animal studies have indicated that supplementation with *Bifidobacteria* might have anti-obesogenic effects [115–118]. Whole grain consumption has been associated with increased abundance of *Bifidobacteria* [119, 120] and a recent study indicated that whole grain rye is superior to whole grain wheat in its ability to increase the abundance of *Bifidobacteria* [59, 121].

3.3 Appetite

The concept of appetite is complex and encompasses a range of different factors that directly or indirectly affect our eating behavior, thus having implications for body weight regulation [122]. Food intake is controlled by both physiological cues, such as stomach distension and appetite-regulating hormones, and psychological cues, such as liking specific foods, which may overrule physiological cues. The physiological concept of appetite has been summarized in the so-called satiety cascade, which was first described by Blundell in 1987 [123] (Figure 3.1). This distinguishes between satiation, which can be described as the physiological cues that determine when we stop eating, and satiety, the physiological cues that appear between meals and determine when we start our next meal. In postprandial appetite studies, appetite often refers to satiety or fullness, hunger and desire to eat, but can in some cases also cover aspects such as prospective food intake, satisfaction and preoccupation with food [124]. The exact wording of questions related to these aspects may vary depending on which language a study is conducted in. For instance, the words satiety and fullness sometimes appear to be used interchangeably in English, whereas they can have distinct meanings in other languages.

Satiation is to a large extent dependent on gastric distension, which occurs as we eat and fill up the stomach [125]. Gastric distension is affected by several factors, such as the volume and viscosity of the food consumed, as well as the gastric emptying rate [126]. A reduced gastric emptying rate extends the time of gastric distension as well as the time for digestion and absorption in the small intestine and can affect the release of gut peptides involved in appetite regulation [127]. Some of the most important gut peptides related to satiety and appetite are glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), peptide YY (PYY) and ghrelin [126]. CCK is released in response to the presence of fat or protein in the duodenum and is thereby involved in the early response to food intake, i.e., satiation. GLP-1 is produced in the small intestine, primarily in response to the presence of fat and carbohydrate, and is thought to play an important role in the so-called ileal brake, which is involved in the control of gastric motility and release of satiety-regulating hormones [126]. GLP-1 is a well-

established pharmaceutical target for treatment of type-2 diabetes due to its ability to stimulate insulin secretion from pancreatic beta cells, and has in recent years received attention as a potential anti-obesity drug due to its appetite-suppressing capability [128–130]. Ghrelin is an orexigenic hormone that decreases immediately after a meal and increases during the postprandial period [131]. Ghrelin has been shown to correlate with subjective hunger ratings and is likely involved in the initiation of an eating occasion [132, 133].

While decreased appetite is often considered an immediate postprandial response to a meal, the effect may extend over several meals, a phenomenon often referred to as the second meal effect. This phenomenon is believed to be mediated through production of short chain fatty acids (SCFAs), i.e., acetate, propionate and butyrate, that are produced through colonic fermentation of dietary fiber [134]. SCFAs have been shown to stimulate secretion of PYY and GLP-1 in animal and *in vitro* studies [135, 136]. This has been confirmed, to a certain extent, in mechanistic studies in humans [137, 138], though the implications of these findings on appetite regulation and food intake remain to be tested.

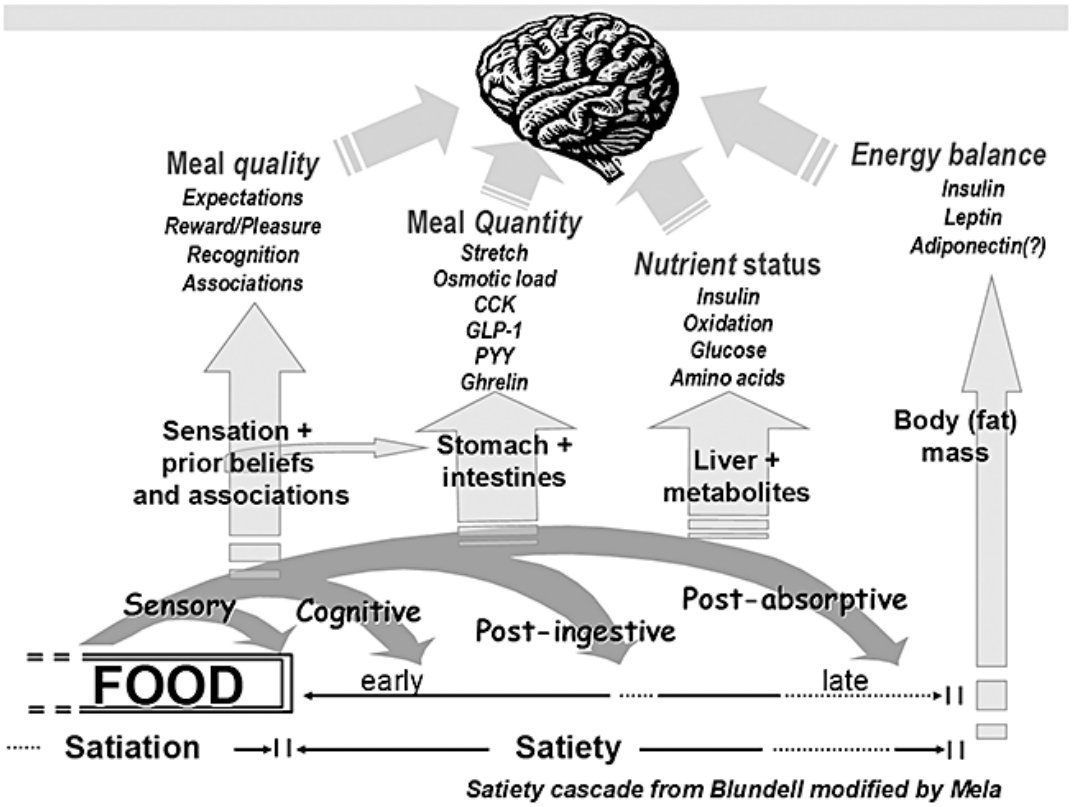

Figure 3.1: The satiety cascade. Reproduced from Blundell et al. 2010 [139] with permission from John Wiley and Sons. Abbreviations: PYY, peptide YY; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1.

3.3.1 Effects of rye consumption on appetite

The satiety-enhancing effects of rye-based products have been well investigated. Acute meal studies have been conducted where subjective appetite has typically been measured at regular time intervals following one or several meals. In these studies, subjective satiety has often been measured by participants rating their sense of fullness, hunger and desire to eat on a visual analogue scale (VAS) anchored with extreme sensations (e.g., "not hungry at all" and "extremely hungry"). Some studies complement this with postprandial blood sampling to measure the concentration of satiety hormones and some studies include an *ad libitum* meal to evaluate whether differences in appetite response translate into differences in subsequent energy intake.

Table 3.2 summarizes randomized cross-over studies investigating subjective appetite response to various rye-based products. The majority of the studies found an improved appetite response after intake of rye-based products compared with control products [140–149]. The rye products included various breads, crisp breads, porridges, kernels and pasta and the rye ingredients varied from sifted rye flour to intact rye kernels. In most cases, the rye products were based on whole grain rye, while the control products were often based on refined wheat. This is likely a reflection of the typical rye and wheat products available to consumers in the countries where the studies were conducted [150, 151] and attempts to study replacement effects aligned with the dietary guidelines. However, the comparisons do give rise to the question of whether the results can be attributed to rye *per se* or are due to other factors, such as dietary fiber.

The results regarding energy intake in a subsequent *ad libitum* meal are inconsistent. Two studies reported a lower energy intake in participants consuming a rye-based breakfast meal [142, 146], while three studies found no difference in energy intake [140, 147, 152]. It is worth noting that two studies that found no effect on energy intake included a standardized lunch meal (second meal) before the *ad libitum* meal, meaning that the time between the rye-based meal and the *ad libitum* meal was longer than in studies not including a standardized lunch meal (480 vs. 240–270 min). This could indicate that a rye-based meal can reduce the energy intake in a subsequent meal, but its effects do not extend to the meal after that.

Results regarding the second meal effect on appetite response are also unclear. Two studies included in Table 3.2 reported that differences in appetite following a rye-based breakfast extended beyond the second meal [141, 144], whereas other studies did not find this [145, 152]. Assessment of the second meal effect often uses the so-called "evening design," where participants consume a test meal for dinner and a standardized breakfast meal (the second meal) on the next morning, followed by postprandial appetite assessment. Also with this study design, results reported in the literature are inconsistent. Sandberg and colleagues conducted a series of studies, of which two studies showed an improvement in appetite response in the morning after a rye-based evening meal, compared with after a wheat-based evening meal [153, 154], though one study using a rye bread with added resistant starch did not confirm this [155]. Lastly, a study from the University of Copenhagen tested the second

meal effect following evening meals with refined grain wheat bread, whole grain rye bread or rye kernels, but found no effect on subjective appetite the following morning [156].

Though the *satiety*-enhancing effects of rye-based products has been relatively well studied, no studies have investigated the *satiating* effects of rye-based products. One study examined the satiating effect of whole grain wheat pasta compared with refined wheat pasta, by having participants consume *ad libitum* meals containing the two types of pasta [157]. The hypothesis was that the whole grain-based pasta would induce increased satiation during the meal and lead to the participants consuming less energy. However, no such difference in energy intake was found in this study [157]. More studies are needed to understand the potential satiating effect of rye and other cereal-based foods and the link to satiety and subsequent energy intake.

Table 3.2: *Overview of randomized cross-over studies investigating subjective appetite in response to rye-based products.*

	Participants	**Intervention products**	**Outcome measures**	**Results** (rye product(s) compared with control product(s) unless otherwise stated)
Isaksson 2008 [140]	N = 22 (64%) A = 21–64 BMI = 18–28	Breakfast meals followed by lunch meals containing (breakfast/lunch): • WG rye porridge / WG wheat pasta (A) • WG rye porridge / refined wheat pasta (B) • RG wheat bread / refined wheat pasta (C)	Subjective appetite (VAS 0–480 min) Lunch served at 240 min. *Ad libitum* dinner served at 480 min (EI measured).	Hunger: ↓A and B (30–380 min) compared with C Satiety: ↑ A and B (30–480 min) compared with C Desire: ↓ A and B (30–480 min) compared with C EI: No difference
Isaksson 2009 [141]	Part A: N = 16 (88%) A = 24–59 BMI = 18–27 Part B: N = 16 (81%) A = 23–60 BMI = 19–26	Breads served as part of a breakfast meal: Part A: • RG wheat bread (control) • Rye bran bread (25 g bran/serving) • Bread with partially milled rye flour (34 g rye flour/serving) • Bread with sifted rye flour (31 g rye flour/serving) Part B: • RG wheat bread (control) • Rye bran bread (25 g bran/serving) • Rye bran bread (16 g bran/serving) • Bread with partially milled rye flour (49 g rye flour/serving) • Bread with partially milled rye flour (30 g rye flour/serving)	Subjective appetite (VAS; AUC 0–240 and 240–480 min) Standardized lunch served at 240 min.	Part A: ↑satiety: before lunch (all rye breads) ↓hunger, ↓desire to eat: before and after lunch (all rye breads) Part B: ↑satiety before lunch (all rye breads), no difference after lunch Hunger and desire to eat: no difference
Rosén 2009 [158]	N = 12 (25%) A = 25±0.8 BMI = 23±0.6	Breakfast meals based on 40 g available starch: • RG wheat bread (control) • WG rye bread • WG rye porridge	Subjective appetite (by bipolar scales from -10 to 10; iAUC 0–180 min)	↑ Satiety (iAUC 0–180 min: WG rye porridge) Hunger: results not reported
Rosén 2011 [159]	N = 20 (50%) A = 21–37 BMI = 22±0.4	Breakfast meals based on 50 g available starch: • RG wheat bread (control) • WG rye bread from variety “Amilo” • WG rye bread from variety “Evolo” • WG rye bread from variety “Kaskelott” • WG rye bread from variety “Picasso” • WG rye bread from variety “Vicello” • Commercial WG rye bread	Subjective appetite (VAS; repeated and AUC 0–180 min)	↓Hunger (AUC 0–60 min: commercial bread, Evolo ; AUC 0–180 min: Evolo) ↓Desire to eat (AUC 0–60 min: commercial bread) ↑Fullness (AUC 0–60 min: Amilo, Vicello; AUC 60–120 min: Evolo)

Rosén 2011 [142]	N = 10 (50%) A = 26±1 BMI = 23±0.4	Breakfast meals based on 50 g available starch: • RG wheat bread (control) • WG rye bread • Wheat kernel • RK	Subjective appetite (VAS; AUC 0–60 min, 120–210 min, 210–270 min, 300–390 min) *Ad libitum* EI (270 min after breakfast)	↑ Fullness (AUC 0–60 min: all test products) ↓ Desire to eat (AUC 0–60 min: Wheat kernel, RK; AUC 120–210 min and 210–270 min: RK) ↓ Hunger (AUC 0–60 min: RK, wheat kernel, WG rye bread; AUC 120–210 min and 210–270 min: RK) ↓ *ad libitum* EI: RK
Rosén 2011 [143]	N = 14 (50%) A = 21–28 BMI = 22±0.5	Breakfast meals based on 50 g available starch: • RG wheat bread (control) • WG rye bread from variety "D.Zlote" • WG rye bread from variety "H.Loire" • WG rye bread from variety "Nikita" • WG rye bread from variety "Rekrut" • WG rye bread from variety "Amilo"	Subjective appetite (repeated and AUC 0–180 min)	↓ Hunger (AUC 0–60 min: D.Zlote and Rekrut; AUC 60–120 min: D.Zlote and Rekrut; AUC 120–180 min: D.Zlote) ↑ Fullness (AUC 0–180 min: all WG rye breads; AUC 0–60 min: D.Zlote and Rekrut; AUC 60–120 min: Nikita, D.Zlote and Rekrut; AUC 120–180 min: D.Zlote, Nikita, Rekrut, Amilo) Desire to eat: no differences
Isaksson 2011 [144]	Part A: N = 24 (92%) A = 20–55 BMI = 19–29 Part B: N = 20 (100%) A = 21–65 BMI = 19–27	Part A: Breakfast meals containing: • RG wheat bread (control) • Milled RK bread • Whole RK bread Part B: Breakfast meals containing: • RG wheat bread (control) • Milled RK porridge • Whole RK porridge	Subjective appetite (VAS; repeated 0–480 min) Standardized lunch served at 240 min	Part A: ↑ Satiety (milled and whole RK breads) ↓Hunger (whole RK, after lunch only) ↓ Desire to eat (whole RK bread) Part B: ↑ Satiety (milled and whole RK) ↓ Hunger (milled and whole RK before lunch, RK only after lunch) ↓ Desire to eat (milled and whole RK before lunch, RK only after lunch)
Isaksson 2012 [145]	N = 24 (79%) A = 33±12 BMI = 23±2	Breakfast meals containing: • RG wheat bread (colored with colorit) (control) • WG rye flakes porridge	Subjective appetite (VAS; repeated 30–720 min) Standardized lunch (240 min), snack (420 min) and dinner (600 min) served	↑ Satiety before lunch ↓ Hunger before lunch ↓ Desire to eat before lunch No difference after lunch
Forsberg 2014 [146]	Part A: N = 21 (52%) A = 39±14 BMI =23±3 Part B: N = 20 (70%) A = 39±14 BMI = 23±3	Part A: Breakfast meals based on containing: • RG wheat bread (108 g) (control) • WG rye crisp bread (80 g) Part B: Breakfast meals containing: • RG wheat bread (86 g) (control) • WG rye crisp bread (64 g)	Subjective appetite (VAS; repeated and AUC 0–240 min) *Ad libitum* EI (240 min after breakfast)	Part A: ↑ Satiety (repeated) ↓ Hunger (AUC 0–240 min and repeated) ↓ Desire to eat (AUC 0–240 min and repeated) *Ad libitum* EI: no difference Part B: ↑Satiety (AUC 0–240 min and repeated) ↓Hunger (AUC 0–240 min and repeated) ↓Desire to eat (AUC 0–240 min and repeated) ↓ *Ad libitum* EI

Hartvigsen 2014 [147]	N = 15 (53%) A = 52–72 BMI = 27–38	Breakfast meals based on 50 g available CHO: • RG wheat bread (control) • RK bread	Subjective appetite (VAS; AUC 0–270 min) *Ad libitum* EI (270 min after breakfast)	↑ Satiety ↓ Hunger ↑ Fullness *Ad libitum* EI: no differences
Hartvigsen 2014 [160]	N = 15 (47%) A = 52–73 BMI = 26–35	Breakfast meals based on 50 g available CHO: • Semolina porridge (control) • RK porridge	Subjective appetite (VAS; AUC 0–360 min) Standardized lunch served at 240 min	No differences in appetite
Johansson 2015 [148]	N = 23 (70%) A = 27–70 BMI = 18–31	Crisp bread served as part of a breakfast meal • 52 g yeast-fermented RG wheat crisp bread (control) • 60 g yeast-fermented WG rye crisp bread • 59 g unfermented WG rye crisp bread	Subjective appetite (VAS; repeated and AUC -30–240 min)	↑ Fullness, both rye breads (AUC and repeated) ↓ Hunger, both rye breads (AUC and repeated) ↓ Desire, both rye breads (repeated) Desire to eat, AUC: no difference
Lee 2017 [152]	N = 21 (48%) A = 23–60 BMI = 21–33	Porridge or RG bread served as part of an isoenergetic breakfast meal • 55 g RG wheat bread (control) • Porridge from 40 g rye flakes (P40) • Porridge from 55 g rye flakes (P55)	Subjective appetite (VAS; repeated and AUC 0–480 min) Standardized lunch served at 240 min *Ad libitum* dinner served at 480 min (EI measured)	P55: ↓ Hunger before lunch, no difference after lunch ↑Fullness before lunch, no difference after lunch Desire to eat and EI: no difference P40: Hunger, fullness, desire to eat and EI: no difference
Zamaratskaia 2017 [149]	N=24 (46%) A = 18–70 BMI = 19–30	Crispbread served as part of a breakfast meal • RG wheat crisp bread (control) • Sourdough-fermented WG rye crisp bread • Unfermented WG rye crisp bread	Subjective appetite (VAS; repeated and AUC 0–360 min)	Repeated hunger, fullness and desire to eat: no difference Fullness AUC: no difference ↓Hunger AUC: sourdough-fermented WG rye ↓Desire to eat AUC: sourdough-fermented and unfermented WG rye

Abbreviations: A, age in years; AUC, area under the curve; BMI, body mass index in kg/m^2; CHO, carbohydrates, EI, energy intake; iAUC, incremental area under the curve; RK, rye kernel; RG, refined grain; VAS, visual analogue scale; WG, whole grain; ↑, increase; ↓, decrease.

3.3.2 The link between appetite regulation and long-term weight management

While a consistent effect of rye-based foods and other whole grain products on subjective appetite has been demonstrated in acute study settings and a consistent association between whole grain intake and body weight has been found in observational studies, the mechanistic link between the acute effect of specific food items on appetite and long-term weight management remains unproven. As previously mentioned, appetite regulation and food intake are results of activities in a complex system that involves both physiological and psychological factors and the ability of a food item to produce increased satiety response in a controlled setting may not translate into long-term reductions in food intake [161]. Indeed, the results on subsequent *ad libitum* food intake following consumption of rye-based products are inconsistent, despite a rather clear satiety-enhancing effect of rye-based products (Table 3.2). However, a recent meta-analysis of studies evaluating long-term weight change (≥ 8 weeks) following exposure to food items that have been shown to enhance satiety found evidence for a link between intake of such food items and long-term weight management, which indicates a link to energy intake [23]. In order to establish a causal link between satiety-enhancing foods and long-term weight loss, appetite assessment should be included in large-scale weight loss studies. However, the commonly used methods for appetite assessment are relatively resource-demanding and there is a need for development of new approaches to appetite assessment adapted for large-scale studies.

3.4 Rye, metabolic risk factors and NCD risk

In addition to the potential link between rye consumption, appetite regulation and body weight management, rye has been linked to several other beneficial health effects such as reduced postprandial insulin [158], reduced cholesterol [59, 162] and reduced levels of low-grade inflammation [163].

Table 3.1 shows that many studies have investigated the effect of cereal intake, especially oats, on blood lipids. It is well established that beta-glucans, present in high amounts in oats and barley, have a lipid-lowering effect at intakes ≥ 3 g per day and the European Commission has authorized health claims on the matter [164–167]. The mechanism behind the effect of beta-glucans on blood lipids is believed to be dependent on binding of cholesterol in the intestine, reducing the re-uptake of cholesterol through the high viscosity of the beta-glucans [168]. While rye does not contain large amounts of beta-glucans, it does contain a rather large amount of soluble arabinoxylans which could be hypothesized to exert a similar effect on blood lipids [22]. Leinonen et al. found that rye bread reduced both total and low density lipoprotein (LDL) cholesterol and found indications of a higher intake of rye leading to a larger decrease in cholesterol levels [78]. On average, the rye breads included in the study by Leinonen provided 3–4 g of soluble fiber per day, which is similar to the amount of soluble beta-glucans considered to have a meaningful effect on blood lipids [164]. A recent study

found indications of a lipid-lowering effect of whole grain rye, but not whole grain wheat, in males with metabolic syndrome, which may be attributed to the higher amount of soluble viscous arabinoxylans in rye, whereas the fiber in wheat is to a larger degree insoluble [59].

Rye has been suggested to have unique effects on postprandial insulin, a phenomenon that has been termed the “rye factor” [22]. The rye factor is characterized by a lowering of the insulin response, without corresponding lowering of glucose, which could indicate an improved insulin economy from consuming rye products [22]. However, in a recent review of the literature on the topic, we concluded that the phenomenon may be attributed to structural properties typical of, but not limited to, rye breads, rather than rye *per se* [Iversen et al., submitted]. This effect is thought to be mediated by several different factors, such as lower gastric emptying rate and digestion and absorption of glucose, which lead to a slower influx of glucose in the blood stream and reduced need for insulin [17, 169–171].

Whole grain and – to some extent – cereal fiber have shown an inverse association with risk markers of type-2 diabetes, such as fasting glucose and HbA1c, as well as with reduced risk of developing type-2 diabetes [17, 39, 172, 173]. Table 3.1 shows that several studies had risk markers related to diabetes, such as insulin sensitivity and glucose tolerance, as their primary outcome [59, 79, 91, 100–103, 110]. However, the results were mixed and the two studies investigating rye-based products found no major effects on fasting or postprandial insulin or glucose [59, 103]. More studies are needed to understand the potential effects of rye consumption on risk markers of type-2 diabetes.

4 METHODS

4.1 Hypotheses and research strategy

Based on suggestive evidence from previous studies, a central hypothesis in this thesis was that consumption of rye-based cereal foods, with whole grains or fractions rich in dietary fiber, could have beneficial effects on body weight and metabolic risk markers. Furthermore, it was hypothesized that this effect might be mediated by mechanisms related to appetite regulation and gut microbiota.

To investigate the effects of rye-based cereal foods on weight loss, a 12-week randomized controlled intervention specifically designed for this purpose was conducted (Paper I). In this study, assessment of appetite was conducted, and fecal samples were collected to investigate the potential links to the main outcomes and various metabolic risk markers were measured (Papers I and II). However, as metabolic risk markers are influenced by weight loss, a study designed for weight loss is not optimal for investigating the effect of rye consumption on metabolic risk markers. Therefore, data were included from a 12-week randomized parallel intervention study, in which metabolic risk markers in the context of weight stability were measured (Paper III). Lastly, a randomized cross-over study investigating the acute appetite response to rye breads with differing amounts of sourdough was conducted. As sourdough is a common ingredient in commercially available rye breads, it was relevant to investigate how this may influence factors believed to be involved in the effect of rye on health (Paper IV). Furthermore, the methodology used in this study was somewhat different from the methodology used in the weight loss study and could highlight some challenges and opportunities in the field of appetite assessment. An overview of the studies, as well as their links to the specific objectives of the thesis, is presented in Table 4.1.

Table 4.1: *Overview of the studies included in the thesis.*

	Papers I and II (the RyeWeight study)	**Paper III (the RyeClaim study)**	**Paper IV (the Sourdough study)**
Design	12 week, 2-armed, parallel, randomized, n = 242	12 week, 2-armed, parallel, randomized, n = 182	Single meal, 6-armed, cross-over, randomized, n = 23
Inclusion criteria	Males and females 30-70 years BMI 27-35 kg/m^2	Males and females 20-70 years 50% with BMI ≤24 kg/m^2 and 50% with BMI>24 kg/m^2	Males and females 20-70 years BMI 18-25 kg/m^2
Intervention	High fiber rye-based cereal products vs. refined wheat cereal products in a hypocaloric context	High fiber rye-based cereal products with fermented rye bran vs. refined wheat cereal products in the context of habitual diet	Breads with varying amounts of whole grain rye and sourdough, compared with refined wheat bread, as part of a breakfast meal
Primary outcome	Body weight and body fat	*Helicobacter pylori* infection	Subjective appetite
Secondary outcomes	Subjective appetite, metabolic risk markers, gut microbiota composition	Metabolic risk markers	Energy intake
Thesis objective(s) and hypotheses	Objectives: A, B, C, D It was hypothesized that high fiber rye would lead to a larger decrease in weight loss and fat loss, as well as improvements in metabolic risk factors, and that such effects could potentially be associated with appetite ratings and gut microbiota.	Objective: D It was hypothesized that high fiber rye products with fermented rye bran consumption would lead to improvements in metabolic risk markers.	Objective: B It was hypothesized that increasing the amounts of sourdough and whole grain rye would lead to improved appetite response and decreased energy intake.

4.2 Study designs and study populations

4.2.1 The RyeWeight study

Papers I and II are based on the RyeWeight study, a parallel, randomized, intervention study designed to investigate the effects of whole grain rye-based foods on body weight and body fat reduction, compared with those of refined wheat-based foods. The study began with a 2-week run-in period where all participants consumed the wheat-based foods, followed by a 12-week parallel phase where participants consumed either rye products or wheat products. During all 14 weeks, participants received guidance from a dietician to reduce energy intake and induce weight loss. The participants underwent a clinical examination three times during the 12-week intervention, at week 0, week 6 and week 12. The primary outcomes of the study were body weight, measured on a digital scale, and body fat percentage, measured using dual energy x-ray absorptiometry (DXA). The study was conducted in Uppsala, Sweden.

The participants included in the RyeWeight study were adults aged 30–70 years with overweight or obesity (BMI 27–35 kg/m^2). Participants were excluded if they had thyroid disorders, type-1 diabetes, history of major GI surgery or chronic GI conditions. Furthermore, people using nicotine products, antidiabetic drugs or anti-obesity drugs were not allowed to participate in the study. Additionally, participants were required to lose at least 0.5 kg during the 2-week run-in period to be randomized to the 12-week parallel phase. Figure 4.1 depicts the flow of participants through the study, with 317 participants starting the run-in phase and 242 of them being randomized to the 12-week parallel phase. During the parallel phase, 14% of the participants dropped out of the study, with the main reasons for dropout being related to difficulties consuming the diet and logistical challenges in fitting study activities into everyday life.

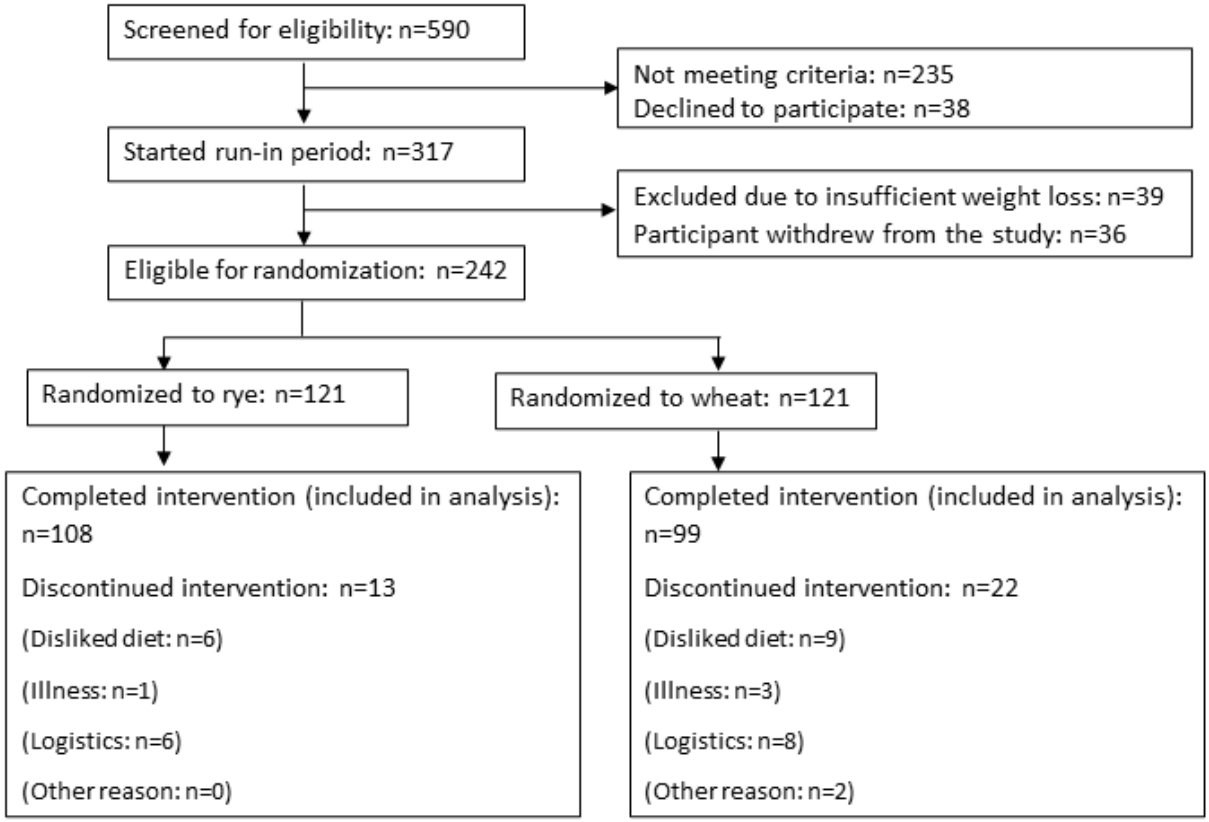

Figure 4.1: *Flowchart of participants in the RyeWeight study (Papers I and II).*

4.2.2 The RyeClaim study

Paper III was based on the RyeClaim study, which was designed to investigate the effects of whole grain rye products with added fermented rye bran on *Helicobacter pylori* infection. A range of metabolic risk markers were measured as secondary outcomes, which were the focus of this thesis. The RyeClaim study was designed as a 12-week parallel intervention where participants were randomized to consume either whole grain rye-based products with added fermented rye bran or refined wheat products for the entire 12-week period. In addition, the participants were invited to a follow-up examination 12 weeks after completing the intervention period (week 24). The study was conducted in Shanghai, China, where the background diet is low in whole grains and particularly low in rye.

As the study was designed to investigate the effect on *Helicobacter pylori* infection, all participants were tested for *Helicobacter pylori* during the screening and a positive test was a requirement for enrollment. Furthermore, participants with active ulcers were excluded. Use of antibiotics or treatment for *Helicobacter pylori* infection were considered concomitant medications and participants using such during the intervention period were not included in the data analysis. The participants were 20–70 years of age and 50% were overweight (BMI ≥ 24 kg/m^2), while the rest were normal weight (BMI < 24 kg/m^2). Smoking or use of medication, except antihypertensives, were reasons for exclusion. In total, 182 participants were enrolled and randomized, of which 161 completed the 12-week intervention. Three of those

participants were excluded from further analysis due to use of concomitant medication during the study, meaning that 158 participants were included in the analysis (Figure 4.2). The main reasons for drop-out were related to travel plans interfering with study participation.

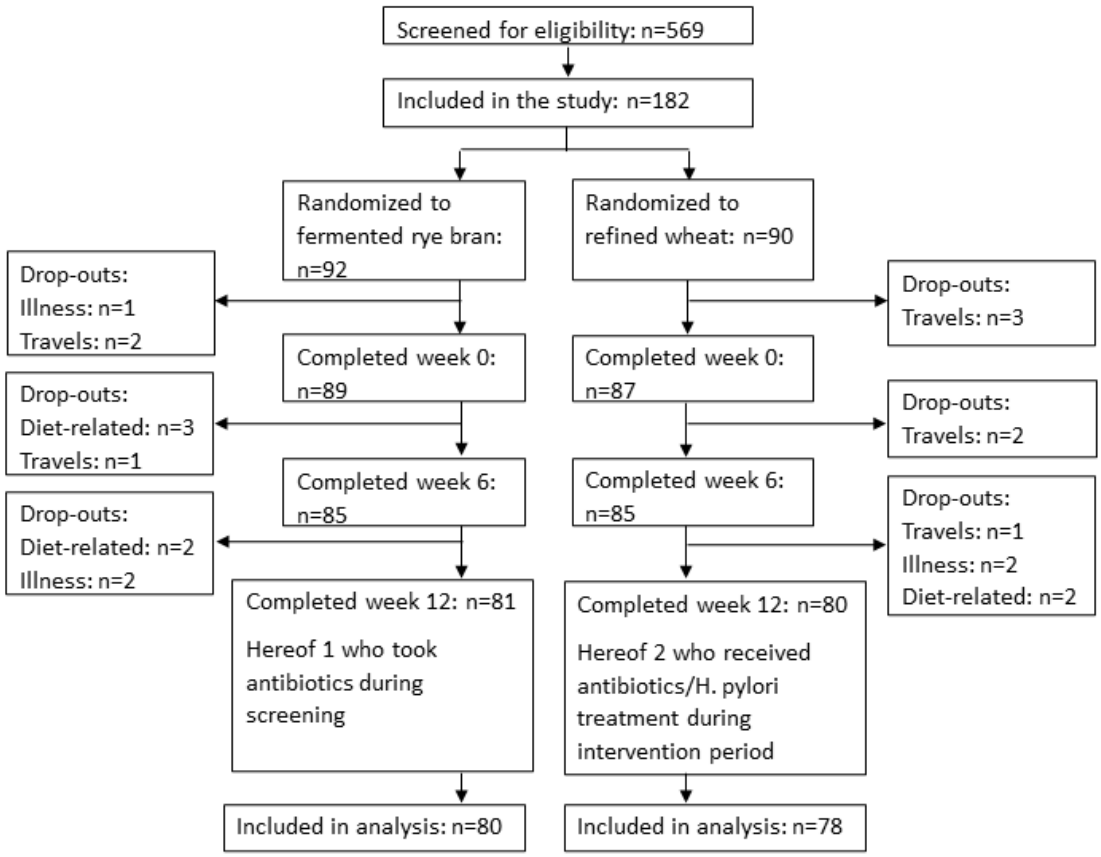

Figure 4.2: *Flowchart of participants in the RyeClaim study (Paper III).*

4.2.3 The Sourdough study

Paper IV was based on a randomized, 6-armed, cross-over study designed to investigate the acute effect of breads with varying amounts of sourdough and rye, compared with refined wheat bread, on subjective appetite. Sourdough is a common ingredient in rye bread and has been hypothesized to influence appetite response. Therefore, this study aimed to investigate the effects of sourdough and rye on appetite response. The participants in the study consumed six test breads (five sourdough rye breads and one wheat bread) as part of a breakfast meal on six separate occasions and recorded their subjective appetite for 4 hours following this meal. The study was conducted in Uppsala, Sweden. The participants were aged 20–70 years, non-smokers with low to moderate physical activity level. In total, 23 participants were included in the study and all of them completed all six test occasions.

4.2.4 Sample size estimation of studies

The sample size for the RyeWeight study was based on a complete case analysis of the two primary outcomes: body weight and body fat percentage. Bonferroni adjustment was applied to account for having two primary outcomes and therefore alpha was set to 2.5%, with a power of 80%. It was determined that 106 complete cases would be needed in each group to detect a 1 kg difference in body weight (standard deviation 2.4 kg) and 1% difference in fat percentage (standard deviation 1.5%). To allow for drop-out rate of up to 18% the inclusion target was 260 participants. The RyeClaim study had a single primary outcome, for which reason the power was set to 80% and alpha to 5%. Based on data from an unpublished pilot study, it was theorized that a 30% decrease in *Helicobacter pylori* load could be of clinical relevance. To allow for a 20% drop-out rate, the plan was to include 90 participants in each group. No formal power calculation was conducted for the Sourdough study; instead, based on previous experiences [148, 149, 152] and power calculations done by Flint et al. [174], it was estimated that 20 complete cases would be needed to enable detection of a difference of 10 % in satiety ratings between treatments.

4.3 Intervention diets

4.3.1 The RyeWeight study

The intervention products in the RyeWeight study consisted of breakfast cereals, crisp bread and soft bread, in both the rye group and the wheat group (Table 4.2). Breakfast cereals consisted of extruded rye and wheat puffs, as well as rolled rye flakes and semolina wheat, packed in 30 g servings. Participants were instructed to consume two packages per day, but could choose freely if they wanted puffs or flakes/semolina. Participants in the rye group had four different rye crisp breads to choose from and were instructed to consume 4–6 slices per day (due to a difference in slice weight between the varieties). The wheat group had only one type of crisp bread and were instructed to consume 5 slices per day. Crisp bread was packed in servings to help participants consume correct amounts and make it easier to take products with them when eating away from home. Frozen soft bread was provided to the participants, who were instructed to eat one serving per day. Participants were given the choice of different breakfast cereal and crisp breads in order to provide some variation that might facilitate compliance and to mimic a real-life setting where people may choose different products depending on their preference.

Participants were instructed not to consume any other cereals than the ones they received from the study, although very small amounts of “hidden” cereals (e.g., sauce thickener) were permitted. The participants filled out a pre-coded compliance journal every day during the study, where they ticked all products they had consumed. Further, participants were instructed to also note down any deviations from the intervention diet, changes in habitual medication, or any illness in the journal. Furthermore, the participants received guidance from dieticians to reduce energy intake and induce weight loss. The dietary guidance followed

a modified version of the Step-wise Weight-determined Accumulative Change Plan (SWAP model) developed by Bertz et al. [175]. The model aims to change each participant's diet in a prioritized step-wise approach by implementing one or more of the following steps: 1) minimizing the intake of sweets, 2) minimizing the intake of fast food, 3) choosing "keyhole" marked products, 4) increasing intake of vegetables and 5) decreasing serving sizes. The aim was to reduce participants' energy intake to an energy deficit of 500 kcal/day, while maintaining an energy source distribution as recommended in the Nordic Nutrition Recommendations [176]. Each participant had an individual meeting with the dietician when initiating the run-in period and was thereafter contacted by the dietician by email or phone approximately 2–3 weeks after their clinical examination in week 0 (4–5 weeks after initiating the dietary intervention).

Table 4.2: *Nutritional composition of the intervention products used in the RyeWeight study (Papers I and II).*

		Product weight (g)	Energy (kcal)	Carbo-hydrates (g)	Protein (g)	Fat (g)	Dietary fiber (g)			Arabinoxylans (g)			Fructans (g)	Klason lignin (g)	Glucose (g)	Total AR (mg)	C17:0/C21:0 ratio
							Total	*Extr.*	*Unextr.*	*Total*	*Extr.*	*Unextr.*					
Per 100 g edible product																	
Rye products																	
Extruded rye puffs		100	345.6	64.0	9.0	2.2	16.78	6.86	9.92	7.16	2.29	4.87	3.85	1.28	3.59	24.9	1.15
Rolled rye flakes		100	345.8	63.7	8.6	2.2	18.33	7.41	10.92	8.34	3.29	5.05	3.02	1.34	4.69	35.8	0.98
Rye crisp bread "Rågi"		100	339.8	65.9	8.8	1.4	14.14	5.60	8.54	6.59	2.47	4.10	2.30	0.65	3.74	39.5	0.93
Rye crisp bread "Husman"		100	335.6	63.1	9.0	1.5	16.95	6.65	10.30	8.16	2.98	5.18	2.45	1.24	4.18	39.7	1.00
Rye crisp bread "Sport"		100	337.5	63.6	8.4	1.6	17.50	6.11	11.39	8.07	2.44	5.64	2.61	1.09	4.80	43.3	0.97
Rye crisp bread "Delikatess"		100	336.1	61.0	10.0	1.5	19.27	7.39	11.88	8.43	2.54	5.89	3.79	1.19	4.96	38.3	0.97
Soft rye bread		100	225.6	34.4	7.5	4.1	10.62	3.75	6.87	4.54	1.50	3.04	1.52	1.04	2.92	32.9	0.86
Wheat products																	
Extruded wheat puffs		100	367.7	73.4	11.5	1.9	5.42	2.51	2.91	2.36	1.24	1.12	1.05	0.78	0.75	0.8	nd**
Wheat semolina		100	353.5	75.0	10.0	0.8	2.95	1.33	1.63	1.17	0.56	0.61	0.62	0.17	0.54	nd*	nd*
Wheat crispbread		100	392.4	65.6	12.4	7.7	5.56	1.52	4.04	2.56	0.86	1.69	0.39	0.50	1.50	4.1	0.05
Soft wheat bread		100	252.2	43.3	13.7	1.9	3.53	1.03	2.50	1.24	0.76	0.49	0.15	0.71	1.05	1.6	nd**
Average daily amount of intervention products, as per protocol																	
Rye	Mean	235	664	114.6	19.2	7.0	32.7	12.3	20.4	14.4	4.9	9.5	5.4	2.6	8.4	80.01	0.93
	(minimum)	(232)	(656)	(112.5)	(18.8)	(6.9)	(30.2)	(11.5)	(18.7)	(13.2)	(4.5)	(8.7)	(4.8)	(2.3)	(7.6)	(75.00)	(0.91)
	(maximum)	(239)	(679)	(117.5)	(19.8)	(7.2)	(34.2)	(12.9)	(21.6)	(15.2)	(5.4)	(10.0)	(6.2)	(2.7)	(9.2)	(86.62)	(0.95)
Wheat	Mean	196	652	118.1	24.2	7.2	8.7	2.9	5.8	3.6	1.6	2.0	0.9	1.1	2..1	4.09	0.03
	(minimum)	(196)	(648)	(117.6)	(23.8)	(6.9)	(7.9)	(2.5)	(5.4)	(3.3)	(1.4)	(1.8)	(0.7)	(0.9)	(2.0)	(3.86)	(0.03)
	(maximum)	(196)	(656)	(118.6)	(24.7)	(7.6)	(9.4)	(3.2)	(6.2)	(4.0)	(1.8)	(2.1)	(1.0)	(1.3)	(2.2)	(4.33)	(0.04)

*no alkylresorcinols detected in the product, **no C17:0 detected in the product.

Abbreviations: AR, alkylresorcinols; extr, extractable; nd, non-detectable; unextr, unextractable.

4.3.2 The RyeClaim study

The intervention products used in the RyeClaim study consisted of whole grain rye crisp bread and whole grain rye extruded breakfast cereals with added fermented rye bran (25% weight/weight of dry ingredients). The rye bran was fermented through a patented process which involves fermentation with *Lactobacillus plantarum* (DSMZ 13890). Supernatant from the fermented rye bran has been shown to reduce *in vitro* adherence and colonization of *Helicobacter pylori* and to reduce the bacterial load in a small pilot study in humans (unpublished data), which was the rationale behind testing the effect of this type of bran product on *Helicobacter pylori* infection in humans on a larger scale. The control group received corresponding crisp breads and extruded breakfast cereals consisting of refined wheat. Participants were instructed to consume a fixed amount of products per day, but were allowed to consume their habitual diet without other restrictions (Table 4.3).

Table 4.3: *Nutritional composition of the products included in the RyeClaim study (Paper III). The amounts listed are what the participants were instructed to consume daily.*

Product	Amount (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Water (g)	Ash (g)
Whole grain rye products with fermented rye bran								
Crisp bread	44.8	145.5	4.3	1.2	23.9	11.1	3.2	1.2
Puffs	110.0	372.6	11.4	3.3	61.2	26.3	5.0	2.8
Sum	***154.8***	***518.1***	***15.7***	***4.5***	***85.1***	***37.4***	***8.2***	***4.0***
Refined wheat products								
Crisp bread	58.8	210.5	6.8	1.0	41.2	3.6	4.5	1.1
Puffs	84.0	302.7	9.7	1.6	58.1	8.6	4.9	1.4
Sum	***142.8***	***513.2***	***16.4***	***2.6***	***99.3***	***12.2***	***9.4***	***2.3***

4.3.3 The Sourdough study

The five sourdough rye breads included in the Sourdough study varied in the amount of sourdough (9, 30 or 51% of dough weight) and rye flour (35, 42 or 48% of dough weight) (Table 4.4). The sourdough consisted of 40:60 whole grain rye flour:water and the flours used for the breads were whole grain rye flour and refined wheat flour. The amount of sourdough was high in the breads with 51% sourdough, compared to most commercial rye breads [151].

The breads were served as part of a breakfast meal containing 100 g bread, 15 g margarine, 20 g cheese, 100 g orange juice and 150 g coffee, tea, or water. Participants stated their preference of coffee, tea, or water on the first occasion and were served the same beverage at all later occasions.

Table 4.4: *Composition, nutritional values, pH and acids of breads used in the Sourdough study (Paper IV). Values are grams per serving (100 g of bread) unless otherwise stated.*

	MS/MR	HS/LR	HS/HR	LS/LR	LS/HR	Refined wheat bread
Sourdough/rye (% of dough weight)	30/42	51/35	51/48	9/35	9/48	0/0
Rye (g)	27	23	30	23	30	0
Protein (g)	7.1	7.2	6.9	7.3	6.3	7.5
Fat (g)	2.2	2.2	2.2	2.2	1.9	3.9
Starch (g)	41.2	41.9	39.9	40.4	39.8	41.9
Total fiber[a] (g)	7.0	6.0	7.2	6.8	8.3	3.6
Soluble fiber (g)	2.3	2.0	2.5	2.1	2.7	1.6
Insoluble fiber (g)	4.7	4.0	4.7	4.7	5.5	3.0
Ash (g)	1.7	1.6	1.7	1.7	1.5	1.4
Water (g)	35.0	35.7	36.1	35.8	35.7	38.0
Energy (kJ)[b]	959	963	934	946	920	992
pH	4.4	4.2	4.2	5.2	5.3	5.0
Lactic acid (g)	0.81	0.81	0.89	0.4	0.36	0.27
Acetic acid (g)	0.13	0.14	0.16	0.07	0.04	0.04

[a] Fiber content as analyzed using the Uppsala method with inclusion of fructans.

[b] Energy content was calculated using a conversion factor of 37 kJ/g for fat, 17 kJ/g for proteins and starch and 8 kJ/g for fiber.

Abbreviations: MS/MR, medium sourdough/medium rye; HS/LR, high sourdough/low rye; HS/HR, high sourdough/high rye; LS/LR, low sourdough/low rye; LS/HR, low sourdough/high rye.

4.3.4 Blinding

Blinding is generally a challenge in dietary interventions, as it is often difficult to conceal from the participants what they are consuming. This was the case in the studies included in this thesis. In the RyeWeight and the RyeClaim studies, intervention products were packed in neutral packaging and marked with neutral codes. However, due to the visual difference between rye and wheat products, it is likely that the participants were able to guess their allocation. In the Sourdough study, the rye breads looked relatively similar, and the participants were not aware that the breads differed in rye and sourdough content. However, the wheat bread still differed visually from the rye breads.

The research staff conducting physical examinations and collecting samples from the participants in the RyeWeight and the RyeClaim study were not aware of the participants' allocation. The participants in the RyeWeight study had their initial consultation with the dietician before randomization, but it is likely that some participants revealed their allocation during follow-up consultations.

4.4 Outcome assessment

4.4.1 Clinical examinations

In both the RyeWeight and the RyeClaim studies, participants underwent a clinical examination in week 0, week 6 and week 12 after an overnight fast. In the RyeClaim study, the examination was repeated in week 24.

In the RyeWeight study, body weight was measured on a digital scale with the participant wearing light clothing, after which waist and hip circumferences, as well as sagittal abdominal diameter, were measured. Blood pressure was measured with an automated blood pressure meter when the participant had been resting in a supine position for 10 minutes, after which venous blood samples were collected. Participants underwent a full body DXA scan to determine body composition, and filled out questionnaires regarding physical activity and GI symptoms.

In the RyeClaim study, participants had a ^{13}C-urea breath test to determine the bacterial load of *Helicobacter pylori*, had their weight and body composition measured on a digital bioimpedance scale and had venous blood samples drawn. A subgroup of participants underwent a DXA scan to validate the body fat percentage measured with the bioimpedance scale.

4.4.2 Appetite assessment

Subjective appetite was measured in both the RyeWeight study and the Sourdough study, but the methodology differed between the two studies.

In the Sourdough study, a "traditional" clinic-based appetite assessment was used, where participants arrived at the research facility in the morning after an overnight fast, answered questions about their appetite and immediately thereafter consumed a breakfast meal. Then, the participants answered questions about their appetite every 30 min for 4 hours. After 4 hours, participants were served an *ad libitum* meal and were asked to eat until comfortably full. The amount of food eaten was recorded to assess whether the food intake at lunch differed depending on the breakfast meal. Between breakfast and lunch, participants could occupy themselves with sedentary activities, such as working on a laptop.

The RyeWeight study had ten times more participants than the Sourdough study, meaning that it was not practically possible to conduct clinic-based appetite assessments. Instead, we designed a protocol for a home-based appetite assessment, where participants conducted the appetite assessment outside the clinical setting. The participants were provided with a standardized meal plan for all meals of the day, as well as a detailed schedule of when to consume which meals and when to answer questions. The meal plans included all meals – breakfast, lunch, dinner, and snacks – with intervention products according to randomization included in the meals. The amounts of food were adjusted to match each participant's energy needs under the hypocaloric conditions which they were generally advised to follow during

the study. All the food needed for each day was provided to the participants. Participants answered questions about their appetite every 30 min from right before breakfast (8:00) until right before lunch (12:00) and then once every hour from 13:00 to 22:00. The participants had dinner at 18:00 and snacks at 15:00 and 20:00.

The questions used to evaluate appetite were the same in both studies. At each time point, participants answered three questions in random order: “How hungry are you?”, “How full are you?”, “How strong is your desire to eat?”. Questions were answered on a 100-point VAS with the following words anchored at each end: “Not hungry at all/I have never felt more hungry,” “Not full at all/Extremely full” and “Not strong at all/Extremely strong”. In the Sourdough study, participants answered the questions on hand-held minicomputers that automatically notified them when it was time to answer questions. In the RyeWeight study, an online survey tool was used to send an email with a link to the questionnaire when it was time to answer questions. In both cases, questionnaires were available on paper, in case of technical difficulties.

4.4.3 Dietary assessment

Dietary intake was measured in the RyeWeight study before the start of the intervention, before the week 6 clinical visit and before the end of the intervention. On each occasion, participants were instructed to complete a 3-day weighed food record. Energy and macronutrient intakes were calculated by a dietician using the software Dietist Net Pro which contains a brand-specific database adapted for use in Sweden. Additionally, intake of foods from different food groups was summarized using the same method as in the national dietary intake survey conducted by the National Food Agency of Sweden, “Riksmaten” [177].

4.4.4 Clinical chemistry

Laboratory work was not included in the thesis work and is described in detail in the papers; it will be described only briefly here.

In the RyeWeight study, blood samples were kept on ice during sampling and were centrifuged and aliquoted immediately after sampling before being stored in a biobank at -80 °C until analysis. Once all participants had completed the study, blood samples were sent to the Department of Clinical Chemistry at Uppsala University Hospital for analysis. Insulin was measured in serum, while glucose, C-reactive protein (CRP), triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol and LDL cholesterol were analyzed in sodium heparin plasma. SCFAs were measured in heparin plasma using a method developed at Chalmers University of Technology [manuscript in preparation]. Alkylresorcinols (AR) were measured in EDTA plasma as a supporting measure of compliance at Chalmers Mass Spectrometry Infrastructure, using a method developed there [178]. The fasting blood samples collected in the RyeClaim study were sent to the laboratory at the Zhongye Hospital

in Shanghai immediately after collection and were analyzed for selected metabolic risk markers. Remaining sample materials were stored in a study biobank at -80 °C and were later analyzed for AR as a marker of compliance in sample extracts taken to the Swedish University of Agricultural Sciences for analysis.

In both the RyeWeight and the RyeClaim studies, total AR was calculated as a biomarker of whole grain intake from rye and wheat sources, while the AR C17:0/ C21:0 homologue ratio was calculated as marker of the proportion of whole grain from wheat and rye sources. Since the C17:0 homologue is primarily present in rye, a higher ratio of C17:0/C21:0 indicates that a larger proportion of the consumed whole grain cereals are from rye, while a low ratio indicates that the intake is primarily from wheat [179].

4.4.5 Gut microbiota composition

Participants in the RyeWeight study collected fecal samples at week 0, week 6 and week 12. Participants were provided a feces collection kit and were instructed to store the fecal samples in a cooling bag with frozen cooling blocks for up to 24 hours before delivering the sample to the clinic, or alternatively store the fecal sample in their household freezer for up to 3 days before transporting it to the clinic in the cooling bag. Samples were stored in the study biobank and after completion of the study, the microbial composition were analyzed using 16S ribosomal RNA at the Swedish University of Agricultural Sciences, as described in paper II.

4.4.6 Data analysis

Statistical data analysis in the RyeClaim study was performed using R Studio, while data from the RyeWeight study and the Sourdough study were analyzed using SAS statistical software. In addition, some figures were created using GraphPad Prism.

The RyeWeight and RyeClaim studies were both randomized, parallel studies and analysis strategy was to compare the two groups at week 6 or week 12, adjusting for baseline (week 0). Analyses of metabolic risk markers were adjusted for change in weight, which is known to affect such markers. The RyeWeight study was a weight loss study and participants lost weight, meaning that the results regarding metabolic risk factors were confounded by this. In addition to adjusting for change in body weight, correlations between changes in metabolic risk markers and changes in body weight were explored; however, confounding cannot be ruled out and results should be interpreted with care. The RyeClaim study was not designed for weight loss and there were no differences between the groups in terms of weight, but metabolic risk factors were not the primary outcomes of the study and some caution should be used when interpreting the results.

The Sourdough study was a cross-over study, which means that each individual served as their own control. An advantage of such design is that it limits the effects of inter-individual

differences. However, it is not optimal for all studies due to the risk of carry-over effect, where the effect of one treatment period can affect the outcome of the following treatment period. Differences in appetite response depending on the breads consumed were analyzed using an analysis of covariance model with a repeated statement to consider the cross-over design. The models were adjusted for appetite response recorded right before breakfast (baseline) as the study was randomized and any differences between the participants' appetite at baseline was assumed to be a result of random variation. This is in contrast to the appetite assessments made in the RyeWeight study, where participants had been consuming the rye or wheat products in the days leading up to the appetite assessment and any differences in appetite before breakfast may have been a result of their regular consumption of such products. It should also be mentioned that the RyeWeight study was not a cross-over study, which means that there has likely been a larger influence of inter-individual differences on the results. However, the larger sample size should have been able to account for this.

5 RESULTS AND DISCUSSION

5.1 Participants, background diet and compliance

Detailed participant characteristics and information regarding diet, compliance and similar have been published in the papers and will be described briefly here. Table 5.1 shows the BMI, age and sex of the participants in the three studies. The RyeWeight and the RyeClaim studies had relatively large sample sizes, compared with many other whole grain interventions (Table 3.1), which was a strength of these studies.

Table 5.1: *Summary of participant characteristics across the three studies reported in Papers I, II, III and IV.*

	RyeWeight (Papers I and II)		**RyeClaim (Paper III)**		**Sourdough (Paper IV)**
	Rye	Wheat	Rye	Wheat	All
n (m/f)	121 (50/71)	121 (45/76)	80 (18/62)	78 (14/64)	23 (8/15)
Age (years)	56.5±9.7	56.9±9.5	44.5±12.5	45.4±14.0	32±9.8
BMI (kg/m^2)	29.7±2.4	30.3±2.4	24.3±3.9	24.1±4.2	22.5±2.7

5.1.1 The RyeWeight study

Dietary records from the RyeWeight study showed that energy intake did not differ between the groups before or during the intervention, but in both groups the energy intake was 100-150 kcal/day lower at week 6 and week 12 compared to the energy intake before the intervention (Paper I). The dietary fiber intake in the wheat group remained relatively stable throughout the intervention, whereas the dietary fiber intake increased in the rye group and was significantly higher than the intake in the wheat group at week 6 and 12 (approximately 20 vs. 38 g/day, Paper I). The protein intake was slightly higher in the wheat group compared with the rye group in week 12, but otherwise the macronutrient intake did not differ between the groups. The physical activity level, assessed through questionnaires, was stable throughout the entire study period (Paper I).

The compliance in the RyeWeight study was good, based on both self-reported product intake (94–95% of prescribed products were consumed on average) and plasma AR, which was used as a supporting biomarker of whole grain rye and wheat intake. In Figure 5.1, a clear separation between week 0 and week 6/12 can be observed in the rye group, while there is no separation in the wheat group, which is what would be expected in a compliant population.

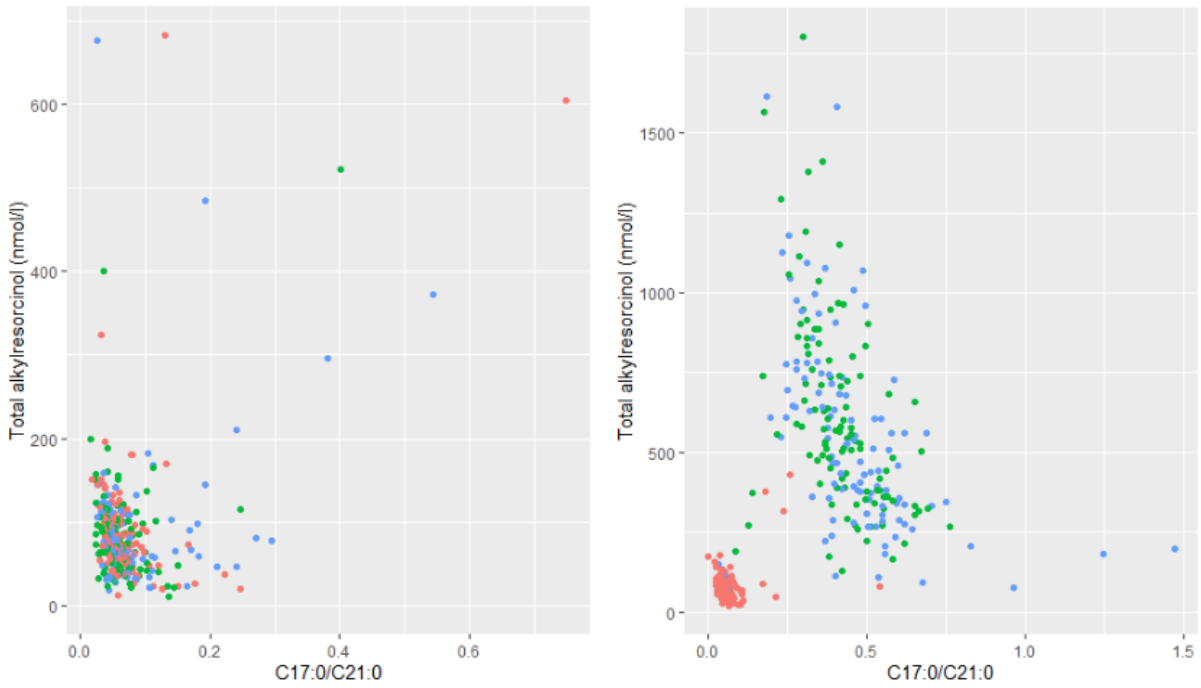

Figure 5.1: *Total plasma alkylresorcinols and the C17:0/C21:0 ratio for the participants in the refined wheat group (left) and the high fiber whole grain rye group (right) in the RyeWeight study. The red points are week 0, green points are week 6 and blue points are week 12. For clarity, two observations (one from each group) with C17:0/C21:0 ≥ 2 have been omitted from the panels. Notice the different axis ranges in the panels.*

5.1.2 The RyeClaim study

Unfortunately, more limited background information was collected in the RyeClaim study and there were no data on background diets or physical activity levels. Compliance was measured through AR and showed an overall poor compliance to the intervention. Figure 5.2 shows that the total plasma AR does not separate the participants from the two groups as clearly as it did in the RyeWeight study. Many participants had C17:0, a marker of rye intake, below the detection limit at baseline, making it difficult to evaluate the change in C17:0/C21:0 ratio over time. The poor compliance could be related to the fact that the type of products used in the study are not commonly consumed in China and that rye has a distinct taste that may not be considered palatable in a Chinese population. However, it should also be noted that there is limited experience of using AR in Asian populations and the results should be interpreted with caution. The total AR concentration in the wheat group in the RyeClaim study was markedly lower than that in the wheat group in the RyeWeight study (2–4 nmol/l vs. 70–80 nmol/l). This could be related to a relatively lower pre-study exposure to AR, as cereals high in AR are consumed to a much lesser extent in China than in Sweden, with lower amounts accumulated in tissue and released into the blood. It has been shown that individuals consuming a gluten-free diet, and therefore having a very low exposure to whole grain rye and wheat, have a very low AR concentration in the blood (< 27 nmol/l) [180]. It should also be mentioned that the samples from the RyeWeight study were analyzed using a liquid chromatography tandem

mass spectrometry-based method which has been shown to overestimate AR concentration in higher concentration range (> 150 nmol/l), compared with the gas chromatography mass spectrometry method that was used to analyze the samples from the RyeClaim study [178]. However, it is highly unlikely that this difference between the methods would explain the 20-fold difference in concentration. There are very few studies reporting AR in Asian populations, but one cross-sectional study investigating the correlation between a specific AR metabolite and type-2 diabetes risk markers found markedly lower plasma concentrations of the specific metabolite in the Asian population compared with a European population, which is likely explained by a lower exposure to AR due to very low intake of whole grain wheat and rye [181]. Nonetheless, the compliance in the rye group overall was low.

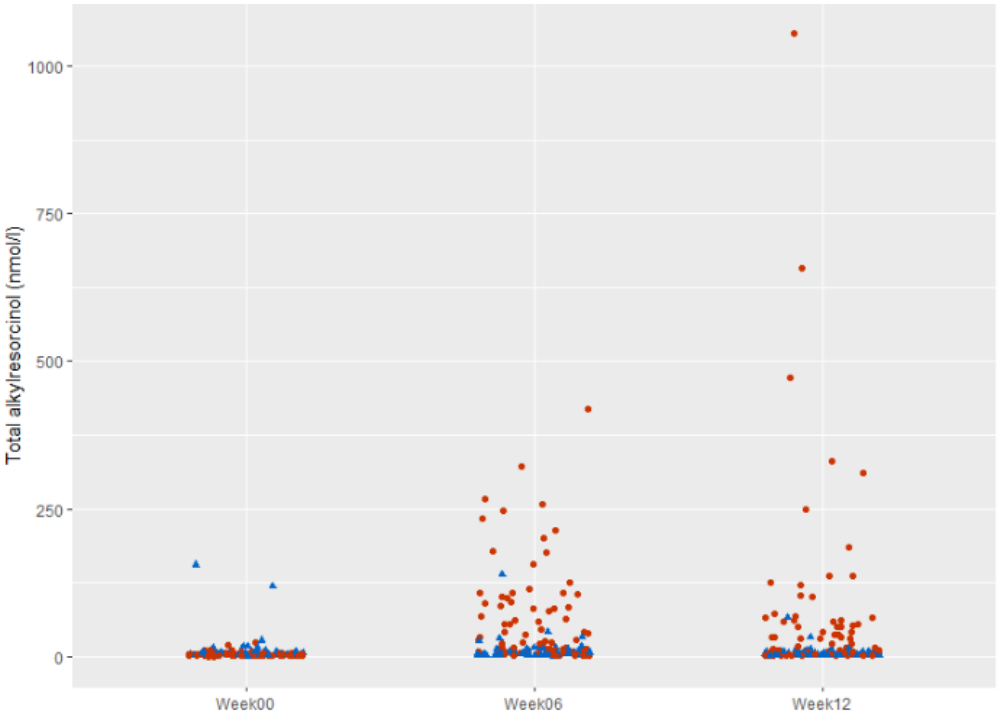

Figure 5.2: *Total plasma alkylresorcinols for participants in the RyeClaim study. Red points are participants randomized to whole grain rye products with fermented rye bran and blue points are participants randomized to refined wheat products.*

5.1.3 The Sourdough study

All participants in the Sourdough study completed all six test meals, except one participant who missed the *ad libitum* meal on one occasion. The participants were not subjected to any interventions or restrictions between the test occasions, so no measures of compliance or similar were included in this study.

When examining the composition of the sourdough breads, we found that the organic acid content, which has been hypothesized to be involved in the mechanism behind improved appetite response following consumption of sourdough bread, was low compared with the

breads with added acid that have been used in several of the studies underlying the hypothesis [182–184]. The sourdough content in the breads used in the Sourdough study was high, up to 51% of dough weight, which produced a relatively sour tasting bread. Furthermore, the amount of acid is high when comparing with breads available on the Swedish market [151], and it seems unlikely that sourdough fermentation alone could produce the amount of acid in some of the studies using added acid to mimic the effect of sourdough fermentation [183, 184]. In conclusion, even though acid may influence appetite, it is unlikely that sourdough fermentation alone would produce enough acid to have a meaningful effect on appetite.

5.2 Effect of rye consumption on body weight and body fat reduction

The RyeWeight study showed that participants in the rye group had a lower body weight than participants in the wheat group after both 6 and 12 weeks of intervention (Figure 5.3). On average, the rye group lost 2.9 kg (of which 2.7 kg were fat) during the 12-week intervention, while the wheat group lost 1.8 kg (of which 1.8 kg were fat). The rye group had a lower body fat percentage at week 12 compared with the wheat group, though this difference was not significant after adjustment for multiple endpoints ($p = 0.031$, significance level: $p < 0.025$). Intention-to-treat analyses confirmed the difference between the groups in body weight and body fat mass, and the difference in body fat percentage was significant in the intention-to-treat analyses, even after adjustment for multiple endpoints (Paper I).

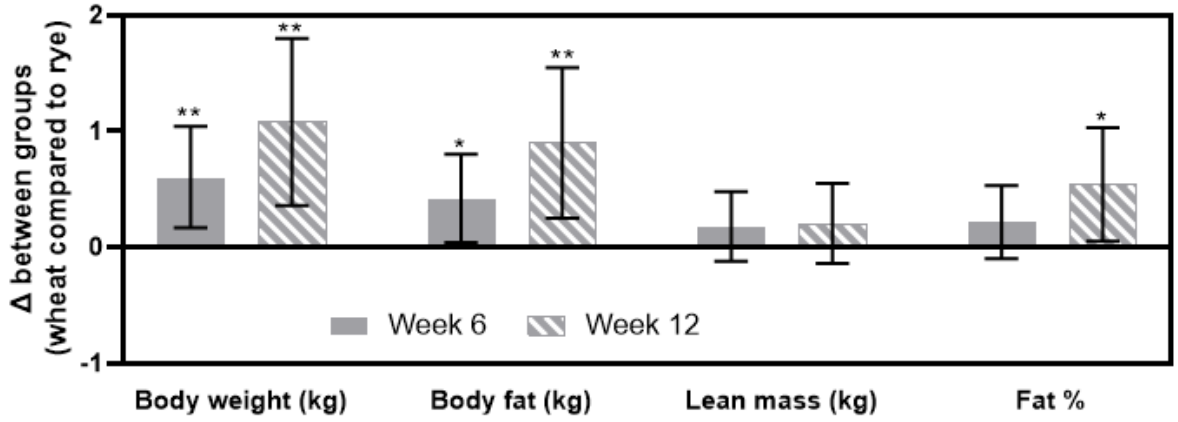

Figure 5.3: *Differences between the rye group and the wheat group in terms of body weight and body composition in the RyeWeight study after 6 and 12 weeks of intervention. The rye group was set as the reference when calculating the difference between the groups, so if the bar reaches above the zero it means that the wheat group was higher than the rye group. * $p < 0.050$, ** $p < 0.001$.*

In the RyeClaim study, there were no differences between the groups in terms of body weight and body fat percentage (Paper III). As this was not a weight loss study, changes in weight were not expected. In fact, as no dietary restrictions were imposed on the participants in the

RyeClaim study, aside from them having to consume the intervention products, it is likely that the products were in some cases added to their habitual diet, rather than replacing other products.

5.2.1 Potential mechanisms behind the effects of rye on body weight and body fat

5.2.1.1 Appetite

Figure 5.4 shows the appetite responses in the appetite assessments conducted in week 0, week 6 and week 12 of the RyeWeight study. Surprisingly, there were no differences between the groups in week 0 and week 12. At week 6, the rye group reported lower desire to eat, lower hunger and higher sense of fullness during the morning period, compared with the wheat group. Over the rest of the day, there were no difference between the groups at week 6. The same was evident when comparing the area under the curve (AUC) (Paper I). There was a difference between the groups at week 6, when assessing AUC for the morning period (8:00-12:00), but there were no differences during the afternoon period (12:00-18:00) or the evening period (18:00-22:00), and no difference when assessing AUC for the whole day. There were no differences between the groups at week 0 or week 12. Correlations between appetite response and changes in body weight and body fat did not reveal any consistent correlations; thus, no indications of a link between subjective appetite response and changes in body weight and body fat in the RyeWeight study were found (Table 5.2).

Previous studies showing a positive effect of rye on appetite response have been conducted in a clinical setting, where participants underwent most of the assessments in a research clinic under controlled conditions (Table 3.2). The RyeWeight study is one of the first studies to conduct appetite assessments at home, in a completely free-living setting. This means that participants have likely been exposed to more outside stimuli, such as seeing or smelling foods not included in the study, talking about food with colleagues and family, and similar things that participants in clinical settings are isolated from. While this has likely led to more variation or "noise" in the data, it has also allowed evaluation of the appetite response under more realistic, real-life conditions [139, 185]. It is important to remember that appetite response obtained under clinical conditions, where participants are isolated from many stimuli they would encounter in everyday life, cannot necessarily be directly transferred to real-life conditions, and extrapolation of results should therefore be done with caution [185]. Studies comparing subjective appetite under free-living conditions and under controlled settings could aid our understanding of this and help us develop a methodology suited for measuring appetite under free-living conditions.

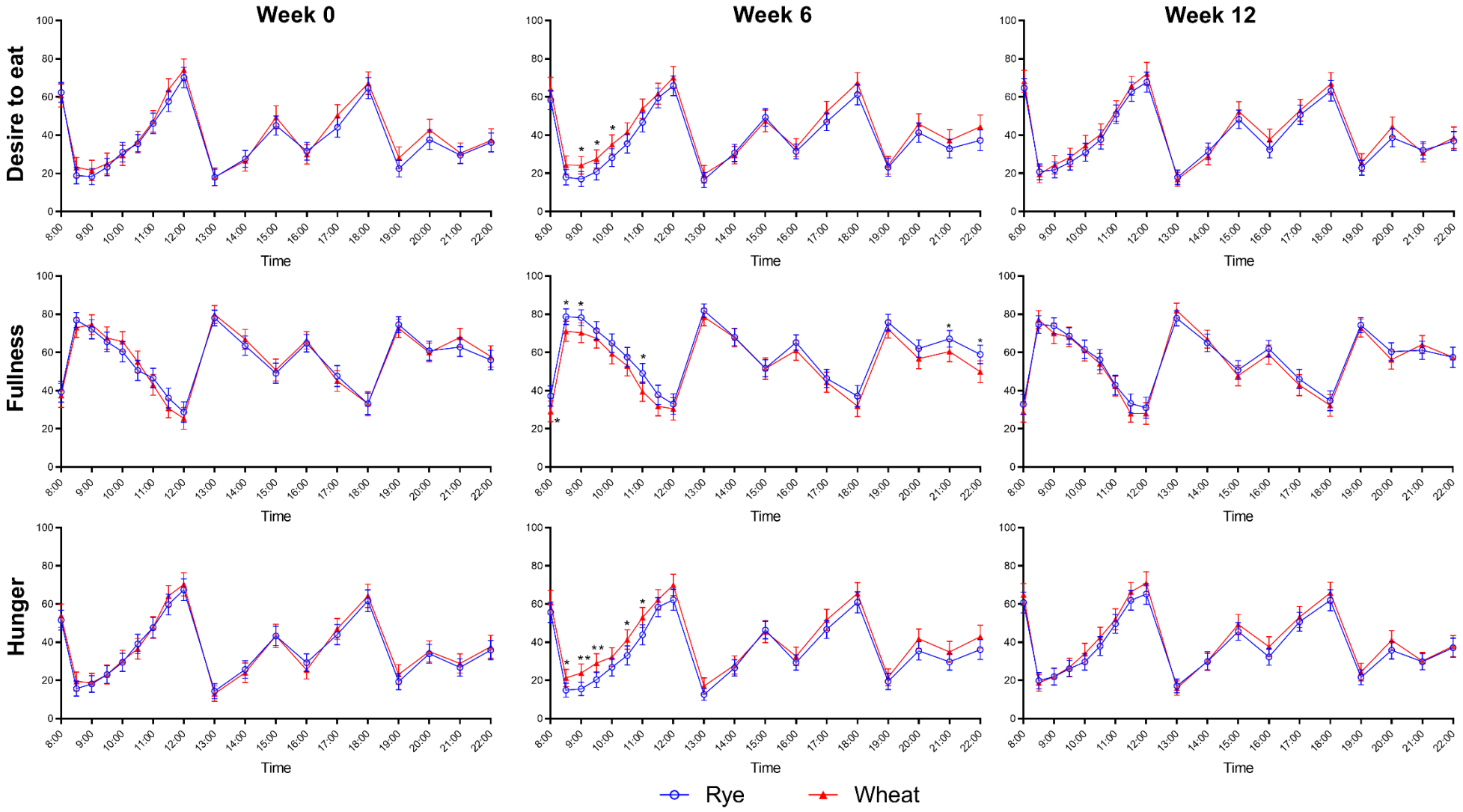

Figure 5.4: *Appetite response in the RyeWeight study. * p < 0.050, ** p < 0.001.*

Table 5.2: *Correlations between AUC appetite response and changes in body weight and body fat in the RyeWeight study.*

	Change week 0 to week 12			Change week 0 to week 6			Change week 6 to week 12		
	ΔWeight (kg)	**ΔFat (kg)**	**ΔFat (%)**	**ΔWeight (kg)**	**ΔFat (kg)**	**ΔFat (%)**	**ΔWeight (kg)**	**ΔFat (kg)**	**ΔFat (%)**
Desire wk0, whole day	0.057 (0.444)	0.009 (0.899)	-0.055 (0.462)	0.123 (0.100)	0.030 (0.690)	-0.054 (0.468)	-0.039 (0.606)	-0.017 (0.819)	-0.030 (0.692)
Fullness wk0, whole day	-0.001 (0.984)	0.026 (0.729)	0.057 (0.441)	-0.0285 (0.703)	0.021 (0.783)	0.0650 (0.384)	0.036 (0.632)	0.028 (0.709)	0.031 (0.681)
Hunger wk0, whole day	0.010 (0.892)	-0.035 (0.642)	-0.095 (0.202)	0,062 (0.402)	-0.017 (0.825)	-0.090 (0.226)	-0.055 (0.462)	-0.045 (0.542)	-0.055 (0.458)
Desire wk6, whole day	0.051 (0.509)	0.024 (0.756)	-0.011 (0.884)	0.114 (0.135)	0.076 (0.322)	0.040 (0.601)	-0.051 (0.509)	-0.043 (0.573)	-0.059 (0.443)
Fullness wk6, whole day	-0.026 (0.736)	0.022 (0.770)	0.072 (0.347)	-0.064 (0.403)	-0.028 (0.718)	0.007 (0.927)	0.037 (0.629)	0.074 (0.336)	0.1081 (0.157)
Hunger wk6, whole day	0.059 (0.446)	0.010 (0.895)	-0.035 (0.6523)	0.102 (0.185)	0.060 (0.437)	0.024 (0.760)	-0.018 (0.813)	-0.048 (0.529)	-0.079 (0.307)
Desire wk12, whole day	0.101 (0.196)	0.041 (0.598)	-0.023 (0.767)	**0.185 (0.017)**	0.079 (0.311)	-0.004 (0.957)	-0.025 (0.750)	-0.010 (0.895)	-0.034 (0.662)
Fullness wk12, whole day	-0.046 (0.555)	0.001 (0.989)	0.052 (0.506)	-0.132 (0.094)	-0.049 (0.532)	0.032 (0.686)	0.063 (0.422)	0.052 (0.511)	0.053 (0.504)
Hunger wk12, whole day	0.109 (0.165)	0.057 (0.470)	0.002 (0.976)	**0.174 (0.026)**	0.093 (0.234)	0.017 (0.833)	0.000 (0.995)	0.002 (0.982)	-0.017 (0.830)
Desire wk0, morning	0.130 (0.075)	0.100 (0.170)	0.041 (0.576)	0.067 (0.364)	0.078 (0.287)	0.030 (0.686)	0.067 (0.364)	0.078 (0.287)	0.02972 (0.686)
Fullness wk0, morning	-0.087 (0.231)	-0.073 (0.318)	-0.032 (0.630)	-0.062 (0.395)	-0.093 (0.203)	-0.059 (0.418)	-0.062 (0.395)	-0.093 (0.203)	-0.059 (0.418)
Hunger wk0, morning	0.098 (0.177)	0.078 (0.281)	0.027 (0.713)	0.069 (0.343)	0.088 (0.226)	0.048 (0.509)	0.069 (0.343)	0.088 (0.226)	0.048 (0.509)
Desire wk6, morning	**0.150 (0.040)**	0.135 (0.064)	0.090 (0.217)	0.054 (0.460)	0.084 (0.254)	0.042 (0.571)	0.054 (0.460)	0.088 (0.254)	0.042 (0.571)
Fullness wk6, morning	-0.128 (0.081)	-0.106 (0.149)	-0.056 (0.442)	-0.074 (0.315)	-0.081 (0.271)	-0.031 (0.677)	-0.074 (0.315)	-0.0809 (0.271)	-0.031 (0.677)
Hunger wk6, morning	0.134 (0.069)	0.098 (0.187)	0.047 (0.527)	0.070 (0.347)	0.056 (0.447)	0.007 (0.927)	0.070 (0.347)	0.056 (0.447)	0.007 (0.927)
Desire wk12, morning	0.107 (0.147)	0.086 (0.242)	0.043 (0.557)	0.005 (0.946)	0.057 (0.444)	0.042 (0.573)	0.005 (0.946)	0.057 (0.444)	0.042 (0.573)
Fullness wk12, morning	-0.062 (0.401)	-0.040 (0.587)	0.007 (0.921)	0.036 (0.623)	-0.027 (0.719)	-0.023 (0.757)	0.036 (0.623)	-0.027 (0.719)	-0.023 (0.757)
Hunger wk12, morning	0.093 (0.206)	0.077 (0.298)	0.042 (0.565)	-0.009 (0.901)	0.050 (0.501)	0.049 (0.506)	-0.009 (0.902)	0.050 (0.501)	0.049 (0.506)

Data are correlation coefficients (*p*-values). Significant ($p < 0.05$) correlations highlighted in bold.

In the Sourdough study, appetite assessments were conducted under more “traditional” conditions. Here, a difference was seen between the refined wheat bread and some of the sourdough rye breads, specifically the ones with high rye content, while there seemed to be no consistent pattern when looking at the sourdough content (Figure 5.5). This was apparent even though the range in rye content was relatively narrow, while the range in sourdough content was relatively wide. One could hypothesize that an even wider range in rye content might have resulted in a stronger effect of rye on appetite. Nonetheless, this study does support an effect of rye consumption on subjective appetite response. On the other hand, the findings from the RyeWeight study could indicate that the improvement in appetite induced by rye products under controlled conditions cannot be transferred to free-living conditions. However, there are also potential weaknesses in the design of the appetite measurements of the RyeWeight study, which could have influenced the results.

While there were generally no differences between the groups in the RyeWeight study in terms of subjective appetite response, there were some indications of differences in subjective appetite response during the morning period at week 6. The fact that the difference was evident during the morning, and not later in the day, could be speculated to be related to the frequency of meals throughout the day. Participants consumed breakfast at 8:00 and lunch at 12:00, leaving 4 hours between meals during the morning. During the afternoon and evening, the participants consumed prescribed meals every 2–3 hours, which might be too often to capture the potentially extended satiety induced by rye consumption. This is in line with observations made while conducting the study, where some participants stated that they felt that the meals were too frequent during the later parts of the day, leaving them little time to develop and experience a clear distinction between hunger and fullness. For future studies, it might be relevant to reconsider the number of meals during the appetite assessment, to capture a wider range of the postprandial state. Additionally, it was difficult for some participants to complete the entire appetite assessment, which was scheduled to finish at 22:00, as it was past their usual bedtime. Therefore, it might be advisable to finalize the assessment at an earlier time in future studies.

Lastly, it should be mentioned that the participants in the RyeWeight study recorded appetite by use of an online survey tool. While this was popular among the participants, who found it very convenient to answer questions on their phones, computers or similar, the specific system used in this study has not been validated for appetite assessment. Traditionally, assessment of appetite response has been done on paper, but recently more studies have been conducted using various electronic systems. Some, but not all of these systems has been validated against paper questionnaires [186–188]. Based on participant feedback on the use of electronic questionnaires, such use should be encouraged in future studies. However, validation against traditional paper questionnaires is needed.

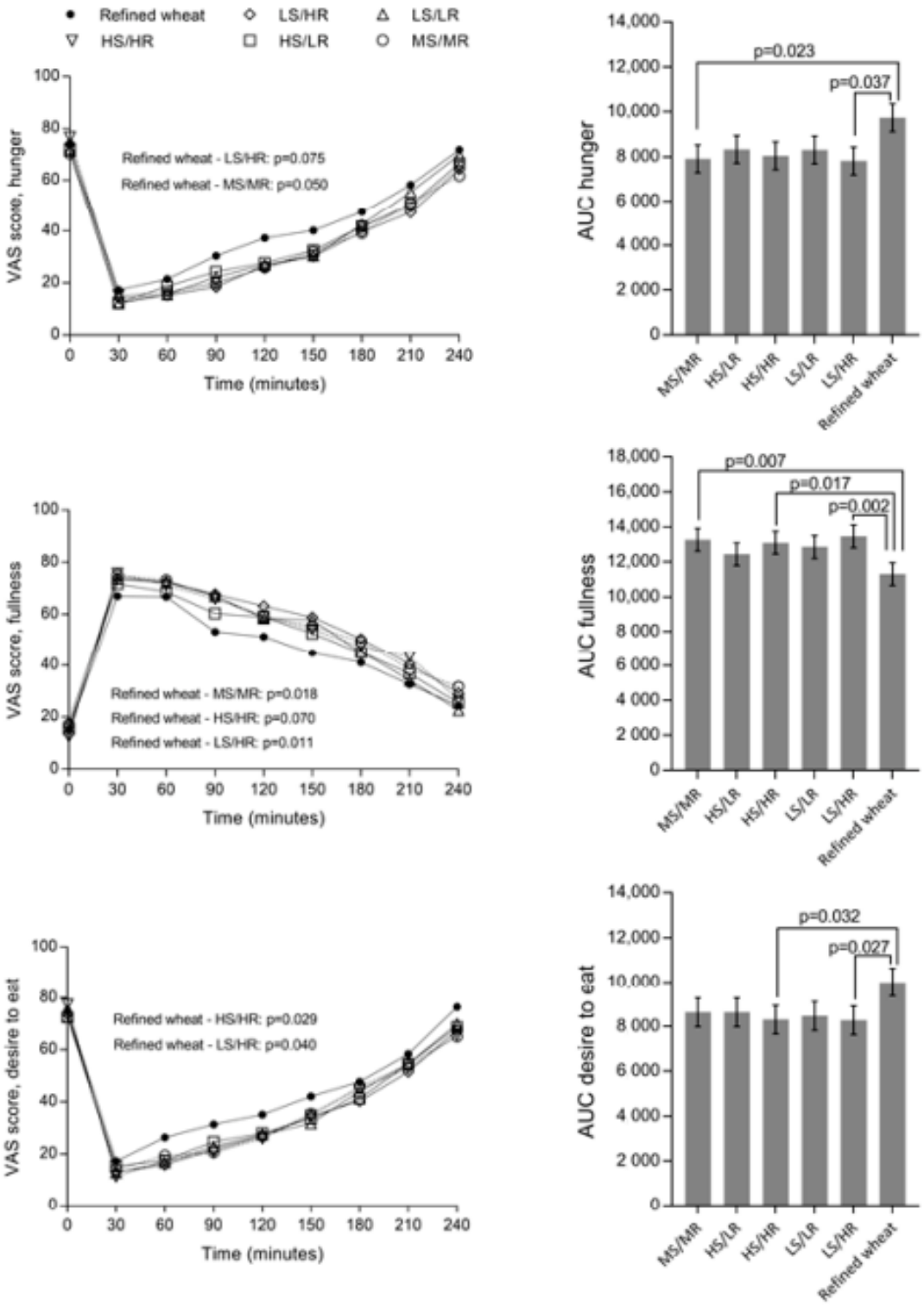

Figure 5.5: *Appetite response in the Sourdough study. Abbreviations: AUC, area under the curve; HS/HR, high sourdough and high rye content; HS/LR, high sourdough and low rye content; LS/HR, low sourdough and high rye content; LS/LR, low sourdough and low rye content; MS/MR, medium sourdough and medium rye content; VAS, visual analogue scale.*

5.2.1.2 Energy intake

The Sourdough study included an *ad libitum* lunch meal, 4 hours after consumption of the breakfast meal. This showed that two of the rye breads resulted in lower energy intake. However, one bread had high rye content and the other low rye content, so this did not suggest that the amount of rye affected subsequent energy intake (Figure 5.6). It should be noted that both breads that resulted in lower energy intake were low in sourdough content, which adds to the notion that a high amount of sourdough does not have a positive effect on subjective appetite response or subsequent energy intake.

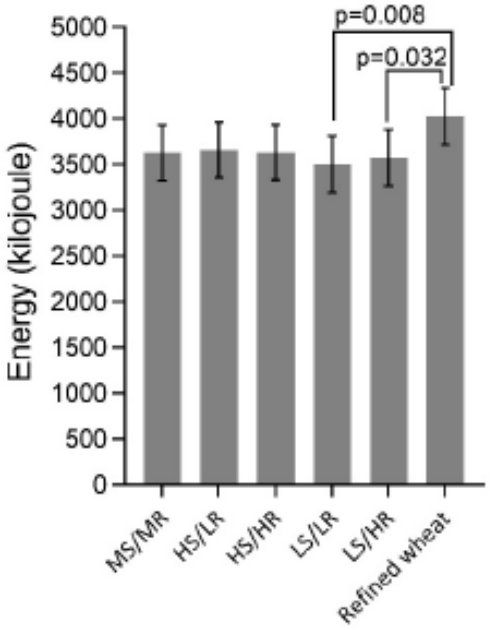

Figure 5.6: *Voluntary energy intake at the ad libitum lunch meal in the Sourdough study. Abbreviations: HS/HR, high sourdough and high rye content; HS/LR, high sourdough and low rye content; LS/HR, low sourdough and high rye content; LS/LR, low sourdough and low rye content; MS/MR, medium sourdough and medium rye content.*

No *ad libitum* meal test was included in the RyeWeight study, as the participants conducted the appetite tests outside the clinical setting. However, data from food records showed positive correlations between change in energy intake and changes in body weight and body fat, indicating that the weight loss was at least to some extent mediated by energy intake (Table 5.3). This is to be expected and does not give any indication as to whether the energy intake was, in turn, mediated by the intervention products. The energy intake did not differ significantly between the groups at any time during the intervention (Paper I). Food records are associated with uncertainties and should be interpreted with caution, but the lack of a difference in energy intake between the rye group and the wheat group may suggest that the difference in weight reduction may in part be rooted in other underlying mechanisms, such as fecal energy excretion and increased energy expenditure [107, 111]. Correlations between

plasma AR and changes in body weight and body fat did reveal inverse correlations between total AR, as well as C17:0/C21:0, and changes in body weight and body fat (Table 5.4). However, this was only evident when correlating data from all participants, irrespective of diet allocations, and may therefore be confounded by the fact that both changes in body weight and body fat and plasma AR differed between the groups. Correlation analysis within the rye and wheat groups did not reveal any significant correlations (Table 5.4).

Table 5.3: *Correlations between dietary intake and changes in body weight and body fat.*

	Change week 0 to week 12			Change week 0 to week 6			Change week 6 to week 12		
	ΔWeight (kg)	**ΔFat (kg)**	**ΔFat (%)**	**ΔWeight (kg)**	**ΔFat (kg)**	**ΔFat (%)**	**ΔWeight (kg)**	**ΔFat (kg)**	**ΔFat (%)**
Δenergy intake wk 0–12 (kcal)	**0.222 (0.001)**	**0.245 (< .001)**	**0.242 (0.001)**	**0.204 (0.004)**	**0.211 (0.003)**	**0.169 (0.016)**	**0.169 (0.017)**	**0.210 (0.003)**	**0.203 (0.004)**
Δenergy intake wk 0–6 (kcal)	**0.185 (0.008)**	**0.225 (0.001)**	**0.230 (0.001)**	**0.220 (0.002)**	**0.247 (< .001)**	**0.208 (0.003)**	0.085 (0.224)	**0.137 (0.050)**	**0.144 (0.039)**
Energy intake wk6 (kcal)	**0.232 (0.001)**	0.134 (0.054)	0.074 (0.290)	**0.219 (0.002)**	0.130 (0.063)	0.074 (0.289)	**0.179 (0.010)**	0.104 (0.137)	0.042 (0.546)
Energy intake wk12 (kcal)	**0.234 (0.001)**	0.126 (0.072)	0.067 (0.341)	**0.169 (0.016)**	0.074 (0.293)	0.029 (0.676)	**0.236 (0.001)**	**0.147 (0.037)**	0.076 (0.278)
Fiber intake wk6 (g)	**-0.287 (< .001)**	**-0.278 (< .001)**	**-0.263 (< .001)**	**-0.238 (0.001)**	**-0.236 (0.001)**	**-0.200 (0.004)**	**-0.241 (0.001)**	**-0.238 (0.006)**	**-0.201 (0.004)**
Fiber intake wk12 (g)	**-0.290 (< .001)**	**-0.258 (< .001)**	**-0.228 (0.001)**	**-0.245 (< .001)**	**-0.210 (0.003)**	**-0.163 (0.020)**	**-0.243 (0.001)**	**-0.231 (0.009)**	**-0.181 (0.010)**
Protein intake wk6 (E%)	0.008 (0.905)	0.011 (0.871)	0.004 (0.956)	0.005 (0.943)	0.008 (0.913)	0.005 (0.945)	-0.003 (0.965)	0.004 (0.9507)	-0.005 (0.942)
Protein intake wk12 (E%)	0.025 (0.723)	-0.006 (0.929)	-0.025 (0.724)	0.039 (0.586)	-0.014 (0.844)	-0.038 (0.592)	-0.013 (0.850)	-0.007 (0.921)	-0.006 (0.930)
Carbohydrate wk6 (E%)	**-0.213 (0.002)**	-0.130 (0.061)	-0.070 (0.321)	**-0.171 (0.014)**	-0.103 (0.140)	-0.045 (0.518)	**-0.188 (0.007)**	-0.119 (0.088)	-0.058 (0.406)
Carbohydrate wk12 (E%)	**-0.301 (< .001)**	**-0.186 (0.008)**	-0.108 (0.124)	**-0.222 (0.001)**	-0.097 (0.171)	-0.005 (0.942)	**-0.295 (< .001)**	**-0.227 (0.001)**	**-0.155 (0.027)**
Fat wk6 (E%)	**0.244 (< .001)**	**0.160 (0.022)**	0.101 (0.148)	**0.199 (0.004)**	0.106 (0.129)	0.040 (0.570)	**0.222 (0.001)**	**0.172 (0.013)**	0.117 (0.095)
Fat wk12 (E%)	**0.283 (< .001)**	**0.174 (0.013)**	0.118 (0.092)	**0.220 (0.002)**	0.075 (0.285)	-0.009 (0.900)	**0.268 (< .001)**	**0.228 (0.001)**	**0.187 (0.008)**

Data are correlation coefficients (*p*-values). Significant ($p < 0.05$) correlations highlighted in bold.

***Table 5.4**: Correlations between change in body weight and body fat and plasma concentration of alkylresorcinols, a biomarker of rye and wheat intake, in the RyeWeight study.*

	All (n = 207)		**Rye group (n = 108)**		**Wheat group (n = 99)**	
	Total AR at week 6	**C17:C21 at week 6**	**Total AR at week 6**	**C17:C21 at week 6**	**Total AR at week 6**	**C17:C21 at week 6**
Δ Weight$_{week\ 0–6}$ (kg)	-0.141 **(0.044)**	-0.139 **(0.046)**	-0.011 (0.909)	-0.085 (0.379)	0.063 (0.539)	0.163 (0.108)
Δ fat mass$_{week\ 0–6}$ (kg)	-0.086 (0.217)	-0.138 **(0.048)**	0.00 (0.994)	-0.117 (0.226)	0.141 (0.166)	0.016 (0.878)
Δ fat %$_{week\ 0–6}$ (%)	-0.053 (0.449)	-0.134 (0.055)	0.015 (0.875)	-0.089 (0.357)	0.162 (0.112)	-0.089 (0.386)
	Total AR at week 12	**C17:C21 at week 12**	**Total AR at week 12**	**C17:C21 at week 12**	**Total AR at week 12**	**C17:C21 at week 12**
Δ Weight$_{week\ 0–12}$ (kg)	-0.188 **(0.007)**	-0.159 **(0.022)**	-0.083 (0.393)	0.058 (0.551)	-0.032 (0.753)	-0.040 (0.699)
Δ fat mass$_{week\ 0–12}$ (kg)	-0.165 **(0.017)**	-0.156 **(0.025)**	-0.069 (0.477)	0.070 (0.474)	0.011 (0.912)	-0.104 (0.309)
Δ fat %$_{week\ 0–12}$ (%)	-0.145 **(0.037)**	-0.175 **(0.012)**	-0.051 (0.599)	-0.001 (0.944)	0.023 (0.821)	-0.148 (0.146)

Data are correlation coefficients (*p*-values). Significant ($p < 0.05$) correlations highlighted in bold.

5.2.1.3 Gut microbiota and SCFAs

In the RyeWeight study, microbiota composition was analyzed and, as reported in Paper II, the intervention induced certain changes in the microbiota. Most notable was an increase in the fiber degrading *Agathobacter* and *Prevotella* in the rye group, although the latter did not differ significantly between the groups due to baseline differences. The increase in these bacteria was likely a response to the high amount of fiber in the rye products, as they are known to degrade fiber and have previously been associated with high fiber intake [189, 190]. Plasma butyrate concentration was higher in the rye group than in the wheat group after 6 and 12 weeks of intervention (Paper II). This may be attributed to the increase in *Agathobacter*, as *Agathobacter* is known to produce butyrate [191]. Acetate differed between the groups after 6 weeks of intervention, but there were no additional effects of the intervention on plasma SCFA concentration (Paper II).

Overall, there was no association between changes in gut microbiota composition and changes in body weight and body fat, aside from an inverse correlation between change in *Holdemania* abundance and change in body fat within the rye group (i.e., increasing abundance of *Holdemania* was associated with reduction in body fat). However, the abundance of *Holdemania* seemed to decrease over the course of the intervention in the rye group, which is counterintuitive. It appears that relatively little is known about the functions

of *Holdemania* in the human gut and its potential implications for human health. Different species and strains of *Holdemania* may have different effects in the human gut. The 16S rRNA methodology used in the RyeWeight study is limited to characterization on a genus level and a deeper characterization may be needed to understand the link between gut microbiota and physiological response to the dietary intervention.

Previous studies have consistently found an association between *Prevotella* abundance at baseline and weight loss in response to a fiber-rich diet [24, 112, 113]. However, no such correlation was found in the RyeWeight study. On the other hand, baseline abundance of *Lactococcus* was seen to correlate negatively with changes in body weight and body fat in the rye group, while correlating positively with the same outcomes in the wheat group. It is important to note that the RyeWeight study included a run-in period and that the baseline fecal samples were collected towards the end of the run-in period and therefore may not reflect the participants' gut microbiota composition before entering the study, since they were provided refined wheat during the run-in. Gut microbiota composition has been shown to change within days in response to dietary intervention, although the dietary interventions used in those studies were rather extreme compared to the intervention used for the RyeWeight study [192, 193]. Nonetheless, conclusions regarding the influence of the baseline microbiota on weight loss should be drawn with great caution. The study was not specifically designed for investigating the effect of gut microbiota on weight loss, but considering recent advances in the field, collection of a fecal sample before initiation of the run-in period would have strengthened the design of the study.

5.3 Effect of rye consumption on metabolic risk markers

Both the RyeWeight and the RyeClaim studies revealed some effects of the intervention on metabolic risk markers. As the RyeWeight study was designed as a weight loss study and succeeded in inducing a weight loss that differed between the groups, the results on metabolic risk markers should be interpreted with caution since it cannot be ruled out that they are to some extent affected by the weight loss.

5.3.1 Inflammation

One of the most interesting findings from the RyeWeight study, beyond the weight loss, was a rather large decrease in CRP (Figure 5.7). CRP concentration remained stable in the wheat group, but decreased in the rye group and was almost 30% lower than in the wheat group after 12 weeks of intervention. The statistical analysis was adjusted for change in body weight and the change in CRP did not appear to be correlated with change in body weight. Thus, this reduction in CRP cannot be explained solely by weight loss, especially considering the fact that this is not the first time rye intake has been associated with reduction in inflammatory markers [194].

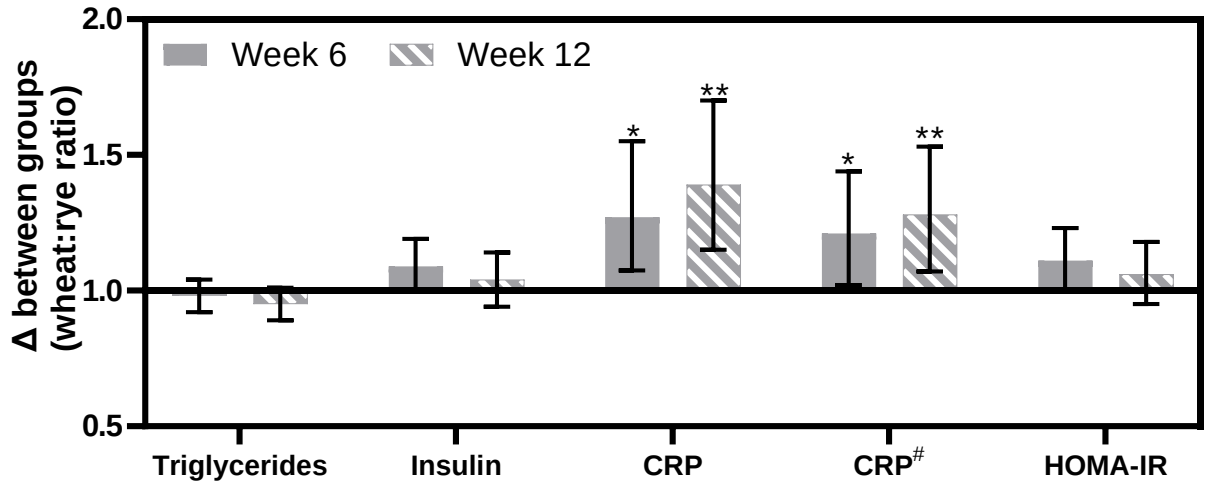

Figure 5.7: *Summary of the difference between the rye group and the wheat group after 6 and 12 weeks of intervention in the RyeWeight study. As the outcome measures were not normally distributed, the data were transformed to a natural logarithmic scale before analysis and the estimates were then back-transformed to the original scale for interpretation. The estimates are therefore to be interpreted as the ratio between the groups and 95% confidence intervals. The rye group was set as the reference and a bar reaching above 1 means that the concentration in the wheat group was higher than that in the rye group. #Observations above 10 mg/l has been omitted* p < 0.05, ** p < 0.01.*

The RyeClaim study also showed a reduction in CRP (Figure 5.8). As opposed to the RyeWeight study, where the difference was driven solely by a decrease in the rye group, the change in CRP in the RyeClaim study was mediated by a combination of an increase in the wheat group and a decrease in the rye group. This may be a result of the products being unfamiliar to the participants, so that any negative effect of wheat products would appear in this population, but not in a population more accustomed to consuming such wheat products, like that in the RyeWeight study. It should be noted that the RyeClaim study was conducted in a population with an ongoing infection (*Helicobacter pylori*) which might affect CRP. However, there was no indication that the infection was affected by the intervention. Furthermore, the differences in CRP had attenuated at week 24, once the participants had returned to their habitual diet, which strengthens the notion that the differences in CRP were caused by the intervention.

Nonetheless, the reduction in CRP was substantial in both studies and a good indicator of a positive effect of rye consumption on low-grade inflammation. This is supported by findings from a cross-over intervention in 17 men with low-grade untreated prostate cancer [194]. In this study, an intervention with high amounts of rye products for 6 weeks tended to decrease levels of CRP and other inflammatory markers that have been associated with metabolic disease risk, compared with refined wheat products [194, 195]. The microbiota analysis from the RyeWeight study revealed a reduction in the abundance of *[Ruminococcus] torques group*

in response to the rye diet (Paper II). *[Ruminococcus] torques group* is known to degrade mucus in the human GI tract and has been associated with poor gut barrier integrity and it may be that the reductions in CRP brought on by the rye-based intervention were mediated through an improved gut barrier function.

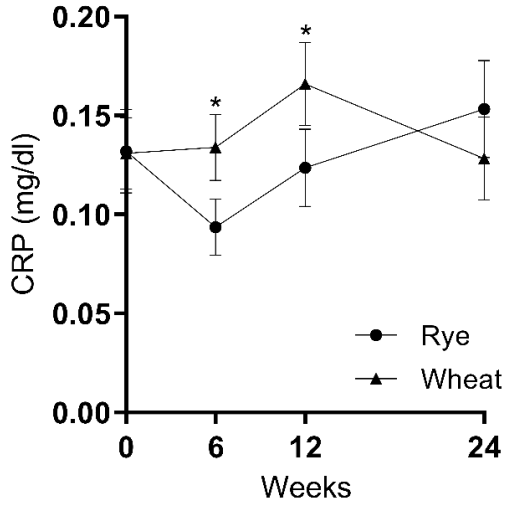

Figure 5.8: *C-reactive protein (CRP) concentration in the RyeClaim study. The participants consumed refined wheat products or high fiber rye products with added rye bran according to randomization during weeks 0-12 whereafter they returned to their habitual diet. Data are means and standard errors of mean. *p<0.05.*

5.3.2 Cholesterol

After 12 weeks of intervention in the RyeClaim study, LDL cholesterol was 0.2 mmol/l lower in the rye group than in the wheat group (Figure 5.9). This was driven by a reduction in LDL concentration in the rye group that was apparent already at week 6. However, at week 6 the LDL concentration in the wheat group had also decreased, meaning that there were no significant differences between the groups at that time. The reduction in LDL cholesterol in the wheat group had diminished at week 12, returning to baseline levels. Interestingly, a seemingly opposite pattern was found in the RyeWeight study, where a difference in LDL concentration between the groups at week 6 was driven by an increase in the wheat group, which had diminished by week 12 (Figure 5.10). These fluctuations in LDL cholesterol concentrations over the course of the intervention are difficult to explain. In a recent intervention comparing whole grain rye and whole grain wheat in an 8-week cross-over study, a significantly lower LDL concentration was found in the rye group after 4 weeks of intervention, but had diminished at week 8 [59]. This was not explained by changes in compliance as measured by plasma AR concentrations. Cereal fiber has been shown to be associated with lower cholesterol concentration and reduced disease risk, while refined grain

consumption has been associated with increased risk of cardiovascular disease and it could be theorized that consumption of high fiber rye-based cereals might reduce cholesterol concentration in a similar manner as oat-based cereals [65, 196, 197]. However, the fluctuations repeatedly seen in cholesterol concentration following rye consumption warrant further research on the topic and highlight the need for studies with a relatively long duration and frequent sampling to capture this phenomenon.

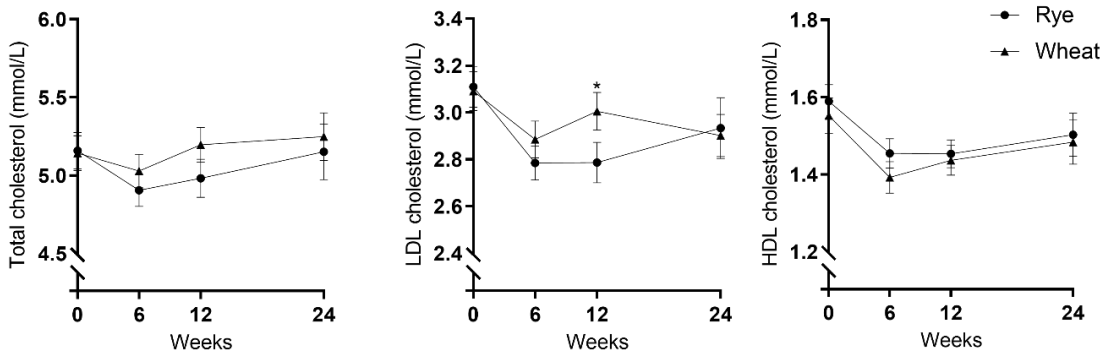

Figure 5.9: *Total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations in the RyeClaim study. The participants consumed refined wheat products or high fiber rye products with added rye bran according to randomization during weeks 0-12 whereafter they returned to their habitual diet. Data are means and standard errors of mean. *p<0.05.*

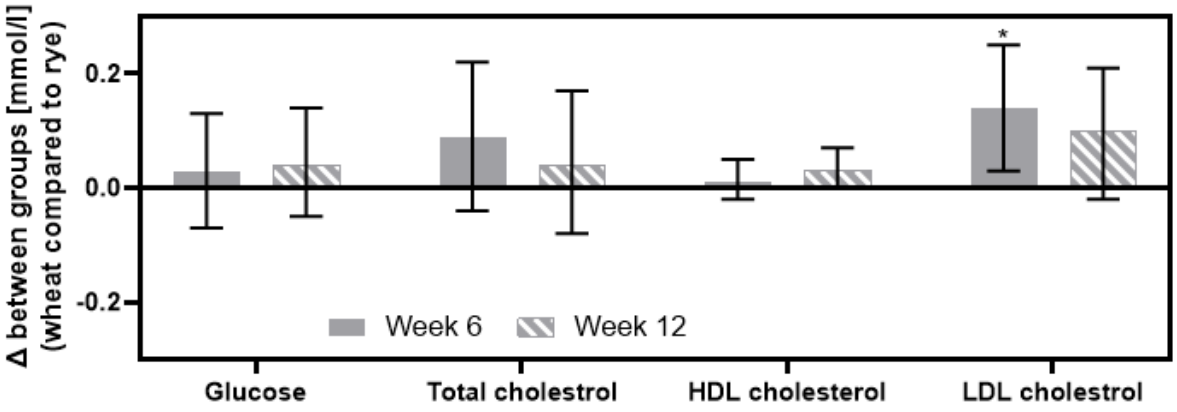

Figure 5.10: *Summary of the mean differences and 95% confidence intervals between the rye group and the wheat group after 6 and 12 weeks of intervention in the RyeWeight study. The rye group was set as the reference when calculating the difference between the groups, so if the bar reaches above the zero it means that the wheat group was higher than the rye group. * p < 0.05, ** p < 0.01.*

6 GENERAL DISCUSSION

The overall aim of this thesis was to investigate the effects of rye-based cereal products on body weight and metabolic risk markers, and the potential roles of appetite regulation and gut microbiota. Rye-based products were shown to induce greater weight loss than wheat-based products, as well as inducing some beneficial effects on metabolic risk markers. In the following, I will discuss some general methodological issues related to the studies included in this thesis, as well as summarizing the main findings of the thesis and how they contribute to further understanding of the health effects of rye.

6.1 Methodological considerations

6.1.1 Choice of intervention products and compliance

All three studies included in this thesis used refined wheat-based products with a relatively low fiber content as the control treatment, whereas the rye-based products contained whole grain and in one study, added rye bran, which made them higher in dietary fiber than the control products. This means that it can be difficult to distinguish the effects of rye *per se* from the effects of cereal fiber, associated bioactive compounds and whole grain.

The rationale behind selection of products, especially in the RyeWeight study, was that the typical rye products on the market are relatively high in whole grain and cereal fiber, whereas the typical wheat products are based on refined wheat. From a public health perspective, it is therefore highly relevant to investigate the effect of replacing existing wheat products with existing rye products rich in whole grains and dietary fiber, in line with the dietary guidelines. This being said, it is still interesting to compare different sources of whole grain to disentangle the potential effects of whole grain vs. refined grain and different cereal sources. Two studies including both a refined wheat arm, a whole grain wheat arm and a whole grain rye arm have been conducted previously [89, 108]. This design gives the opportunity to investigate the effect of whole grain vs. refined grain, as well as the potential differences between cereal sources, but both studies had a relatively low number of participants in each arm and thus had limited statistical power to detect clinically meaningful differences. Though it could have been interesting to include another treatment arm in the RyeWeight study, for instance with whole grain wheat-based products or refined rye products, it would have had some implications: either the power would have been reduced or there would have been a need to increase the enrollment, which can be a challenge in practice. Furthermore, one of the previously mentioned studies indicated that whole grain rye was superior to whole grain wheat in terms of inducing weight loss [108], which would justify the choice of comparing whole grain rye with refined wheat at a larger scale.

Another option could be to exclude the refined wheat arm and only include two different whole grain groups. A study by Eriksen et al. compared whole grain rye and whole grain wheat, matched for cereal fiber content by adding wheat bran to the whole grain wheat products [59]. This gave the opportunity to investigate the effect of the whole grain source, without the influence of differing amounts of cereal fiber, and indicated that rye was superior to wheat in terms of lowering LDL cholesterol, independent of fiber content [59]. However, it is important to remember that different cereals have different amounts of fiber in their native form [81], so in practice a 100% whole grain rye product will have a higher fiber content than a 100% whole grain wheat product. Ultimately, choice of control treatment is a challenge in dietary intervention studies and should be made carefully, to fit the research question [198, 199].

In the context of the RyeClaim study, neither the rye products nor the wheat products were similar to any products available in the market or habitually consumed in the population where the study was conducted. However, the aim of this study was to proof a concept of using a combination of whole grain rye and fermented rye bran to manage *Helicobacter pylori* infection, rather than testing a diet with immediate potential for utilization. As rye is generally not consumed in countries with a high *Helicobacter pylori* incidence, it was necessary to conduct the study in a population that was not used to consuming rye-based products and unfamiliarity with the products was thus difficult to avoid. In retrospect, the poor compliance indicates that more care could have been taken to ensure that the intervention products were palatable for the participants. This highlights an important aspect of designing dietary interventions, that should not be neglected. Good compliance is an important factor for producing robust evidence [75, 200], so one should take measures to facilitate compliance and ensure that the intervention is well accepted by the participants. Furthermore, this also highlights the importance of measuring compliance in intervention studies [201]. Compliance is often measured by having the participants report their intake of intervention products or by having participants return unused intervention products [202, 203]. However, these measures are prone to bias and should ideally be supplemented with more objective measures, such as dietary biomarkers – which were used in the studies included in this thesis. Without dietary biomarkers, lack of compliance would not have been detected in the RyeClaim study.

While rye breads are often made using sourdough, the breads used in the RyeWeight and RyeClaim studies were made without sourdough, to prevent any potentially confounding effect of comparing yeast-fermented wheat breads with sourdough-fermented rye breads. The Sourdough study showed no effect of sourdough fermentation on postprandial appetite. However, sourdough could have other effects, for instance related to bioavailability and structural properties of the bread, that may affect health outcomes [204]. Furthermore, sourdough can be based on a range of different cereal ingredients and yeast cultures and effects may vary between different types of sourdough [204].

6.1.2 Appetite assessment

It was hypothesized that weight loss induced by rye consumption could be mediated through increased satiety. This was not confirmed by the studies included in this thesis, but methodological issues may have affected the results.

There are currently few studies that have conducted home-based appetite assessments and therefore little is known about the validity of such measurements, compared with the traditional clinic-based appetite assessments. One study comparing appetite response to whole grain-based products in a clinical setting with that in an at-home setting found appetite response in the two settings to be comparable [205]. However, more studies are needed to validate the home-based approach. Home-based appetite assessments can probably provide valuable insight into appetite regulation under free-living conditions and help us understand how the body of evidence from clinic-based studies translates into real-life settings. Furthermore, home-based appetite assessment offers practical advantages in terms of reducing the need for clinical facilities to conduct appetite assessments and participants may also find it appealing to conduct study activities outside the clinical setting. This feeds into an ongoing trend within the field of medical research where decentralized and virtual trials are gaining momentum [206–208].

6.2 Rye and health – evidentiary status

6.2.1 Body weight management

Studies in this thesis have provided evidence that rye has a beneficial effect when it comes to reducing body weight and body fat, but the mechanism(s) behind this effect remain unclear and warrant further research.

Considering the large body of studies showing a satiety-enhancing effect of rye-based products (Table 3.2), it is worth considering appetite regulation as a potential mechanism, even though no such mechanistic link was found in the RyeWeight study. As previously discussed, the methodology used to evaluate satiety in a home setting needs to be optimized and validated and the link between inducing satiety and inducing weight loss should be investigated further. In addition, the potential role of satiation is worth considering. No study to date has investigated the satiating effect of rye-based products, but from a theoretical point of view, satiation induced by rye-based products could lead to a lower energy intake within a meal, which could contribute to lower overall energy intake and, consequently, weight loss [125]. Additional mechanisms, such as fecal energy excretion and energy expenditure, could also be worth including in future studies. Studies in ileostomy patients have shown increased energy content in the ileal digesta following consumption of rye products, compared with wheat products, which indicates that a lower proportion of the energy in the food is absorbed in the upper GI tract when rye products are incorporated in

the diet [209, 210]. No studies have measured fecal energy excretion following a rye-based diet, but consumption of whole grain and dietary fiber has been shown to increase fecal energy excretion [211–213]. Thus, it seems plausible that rye products would have the same effect, but this remains to be tested.

As gut microbiota, especially baseline gut microbiota, has recently emerged as a potential mediator of weight loss induced by a fiber-rich diet, future studies should consider this [25]. Energy extracted from fermentable dietary fiber in the colon, in the form of SCFAs, has previously been thought to be a significant source of energy and thus shift the energy balance upward [214]. However, the fact that intake of dietary fiber has consistently been associated with lower body weight and that concentrations of certain fiber-degrading bacteria have recently been correlated with increased weight loss in response to a fiber-rich diet speaks against that theory [24, 112, 113]. Rather, it may be that SCFAs, especially propionate, are involved in appetite regulation, through their potential effect on satiety-regulating hormones, which could explain why a high abundance of SCFA-producing bacteria is associated with increased weight loss following a diet high in dietary fiber [121, 135, 215, 216]. This could mean that a person with a higher capacity for colonic fermentation and SCFA production would respond better to a weight loss diet high in fermentable fiber than a person with lower capacity for fermentation. Butyrate was the only SCFA for which the concentration differed consistently between the groups in the RyeWeight study. Butyrate has been suggested to be involved in the upregulation of the energy expenditure and it could be that the increased weight loss in the rye group was partially mediated by this, especially considering the fact that neither reported energy intake or subjective appetite differed between the groups [217]. In future studies, it could be interesting to include a measure of energy expenditure, though it is relatively resource-demanding to perform such measurements in a large group of participants [218].

The lack of a “true” baseline fecal sample in the RyeWeight study hampered investigation of the potential link between baseline gut microbiota and response to the intervention, since the 2-week run-in period on refined wheat may have altered the baseline gut microbiota. On the one hand, some studies have shown that gut microbiota composition changes rapidly in response to dietary change [192, 193]. However, previous cereal-based interventions similar to the RyeWeight study have shown limited effects of the intervention on gut microbiota composition [57–59, 219]. A solution might be to conduct a model intervention of the run-in period, using the same dietary intervention as was used in the RyeWeight study, to increase our understanding of how the run-in period may have affected the microbiota composition and how the data from the RyeWeight study should be interpreted.

The RyeWeight study is the first study designed to investigate the effect of rye consumption on weight loss and though the results are convincing, the findings should be confirmed in other studies before firm conclusions are drawn. While the RyeWeight study was of a relatively long duration compared with other whole grain interventions (Table 3.1), it is important to consider the more long-term effects of a weight loss intervention. Weight gain

following weight loss is far from unusual and long-term weight management is the most important aspect to consider for disease prevention [220–222]. Future studies could have extended intervention periods, but follow-up periods beyond the intervention period could also provide valuable information on the long-term effects of weight loss induced by replacing refined wheat-based cereals with whole grain rye-based cereals.

6.2.2 Metabolic risk factors

A consistent effect of rye consumption on inflammation was found in this thesis and a potential association with gut microbiota was revealed. This is consistent with findings of another rye-based intervention, showing that a high intake of rye-based products reduced levels of several inflammatory markers [194]. It is important to emphasize that none of these studies was designed for investigating the effect of rye on inflammation and each has some potentially confounding factors, such as weight loss and ongoing disease. Despite this, the results are consistent and a potential mechanistic link to gut microbiota has been suggested, which would justify designing and conducting a study specifically to investigate the effects of rye-based products on inflammation. In such a study, it would be relevant to include assessment of other inflammatory markers that may have implications for metabolic and cardiovascular disease risk, such as interleukin-6 and tumor necrosis factor-α [223]. Furthermore, it could be relevant to include markers of gut barrier integrity, such as zonulin [224], in order to investigate the potential association between inflammation and the gut [225]. There is no consensus when it comes to cut-offs for CRP in relation to risk of cardiovascular disease, but it has been suggested that values of 1–3 mg/l should be interpreted as intermediate risk and values > 3 mg/l as high risk [226, 227]. Participants in both the RyeWeight and the RyeClaim studies had a baseline CRP of approximately 1.5 mg/l, placing them in the intermediate risk group. In both studies, the CRP concentrations in the rye group at week 12 were close to the lower limit of 1 mg/l, indicating a reduction in disease risk, although it should be noted that they were in the lower range of the intermediate risk group already at baseline.

The studies included in this thesis revealed a potential effect of rye consumption on LDL cholesterol. Although not as clear-cut as other results, this raises interest, as it is not the first time rye-based products have been associated with favorable effects on blood lipids [59, 78, 228]. Though the participants in the RyeClaim study had an average LDL concentration within normal range at baseline, the magnitude of the reduction was in line with what has been seen in studies investigating the cholesterol-lowering effect of beta-glucans, which has been deemed sufficient to substantiate an authorized health claim from the European Commission [164, 167]. However, at this stage, the results are not sufficiently consistent to draw any conclusions regarding the potential effects of rye consumption on blood lipids.

7 CONCLUSIONS

The work in this thesis showed that:

- Rye-based products with a high fiber content induce a larger weight loss than refined wheat products, which indicates that replacing refined wheat products with high fiber rye products is beneficial for weight loss.

- The effects of rye-based products on appetite were not convincing and the appetite response did not differ between high fiber rye-based products and refined wheat-based products in an at-home setting. No mechanistic link between increased satiety and weight loss could be established. However, methodological issues warrant further research before any conclusions can be drawn.

- No consistent link between weight loss and gut microbiota was found, but use of more sophisticated analysis methods and a better understanding of how the run-in period may have affected the baseline gut microbiota could potentially lead to additional findings.

- Rye was shown to induce a consistent attenuating effect on inflammation, compared with refined wheat products, though these results should be interpreted with care due to potential confounding. Rye may affect LDL cholesterol, but the effects were inconsistent and warrant further research. Changes in gut microbiota induced by rye consumption may be involved in the reduction of inflammation.

8 FUTURE PERSPECTIVES

- Confirmation of the effect of rye on body weight in other studies is warranted, especially if one were to pursue a health claim on the effect or in other ways use it in marketing of rye products.

- Long-term weight maintenance is an important aspect of weight management and follow-up studies, for instance 1–2 years after the end of an intervention, would aid the understanding of the long-term implications of a weight loss induced by replacing wheat products with rye products. Studies of longer duration and studies including a weight maintenance phase, in addition to a weight loss phase, could also provide valuable information on the potential use of rye for weight maintenance.

- Further investigation of the potential role of gut microbiota, potentially through sequencing of gut microbiota on a deeper level, more advanced statistical analysis methods and carefully planned sample collection, could likely increase our understanding of some of the underlying mechanisms of weight loss induced by a dietary intervention.

- Methods suitable for measuring subjective appetite under free-living conditions should be developed. This could for instance be done by comparing the home- and clinic-based appetite responses to identical foods in order to improve our understanding of how we can interpret appetite responses measured in different settings and identify aspects of the methodology that can be optimized for these different settings. Furthermore, there is a need to develop and validate new tools for appetite assessment, such as a smartphone application.

- The effects on inflammation warrant further research. A first step could be to utilize biobank material from the RyeWeight study to measure additional inflammatory markers, as well as markers of gut permeability. At some point, a study specifically designed to investigate the effect of rye consumption on inflammation, without the confounding factor of weight loss, will be needed to establish causality.

- A study designed to investigate the effect of rye consumption on inflammation could potentially also be utilized to investigate the effect on blood lipids. Ideally, relatively frequent sampling (e.g., every second week) should be implemented to capture the fluctuations in LDL cholesterol we have seen on several occasions.

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